UNIVERSITY OF EDINBURGH

THESIS

entitled

THE EFFECTS OF LACTATION UPON CERTAIN CONSTITUENTS OF BLOOD, MILK, AND RUMEN LIQUOR

submitted

by

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C O N T E N T S

INTRODUCTION

The mammary gland of the lactating cow synthesises milk from a variety of substrates carried to it in the blood stream. The substrates may be derived from direct absorption of the end products of digestion in the alimentary tract, or these may be modified in the liver or in their passage across the gut epithelium before passing into the peripheral blood supply.

There is a considerable body of evidence to show that acetic acid and beta-hydroxybutyric acid are used by the lactating mammary gland for the synthesis of fatty acids and thus for milk fat production. Propionic and butyric acids can make a small contribution to fat formation, the former as a source of acids with an odd number of carbon atoms, and the latter as a source of preformed two carbon fragments for the mammary gland. These three fatty acids, along with others, are produced in the rumen. A large volume of work has shown that the amounts and proportions of the acids in the rumen contents vary considerably and that these variations can influence the composition of milk, particularly its fat content. Beta-hydroxybutyric acid is also a normal constituent of rumen contents along with acetone and acetoacetic acid. The acids and ketones are present in blood and a few workers have attempted to relate blood levels with rumen liquor levels on the one hand and with milk production on the other.

Lactation begins at calving, continues through the active milk-producing phase and ends with the cessation of milking or drying off. Parturition itself subjects the animal to considerable stress and subsequently she is faced with the enormous biochemical and

physiological task of mobilising the raw materials and energy for the extensive synthetic activities of the mammary gland. During the main milk-producing period milk yield and composition vary and so do the demands of the mammary gland for milk production. At drying-off these demands cease but the effect of cessation is cushioned by the fact that milk production has been falling and has been at a low level for some time. The far reaching effects of parturition, initiation of lactation and drying-off would be expected to manifest themselves in, or be accompanied by, changes in the blood levels of metabolites concerned in the synthetic activities of the mammary gland. The relationships between the blood levels of certain of these metabolites and changes in milk yield and composition, associated with lactation, form the major part of this investigation.

In order that the changes in milk yield and composition should be true lactation changes, the effect of other factors had to be eliminated as far as possible. The cows under experiment were thus maintained in a constant environment in a byre, and with a constant milking routine. Control over udder infections was very strict and the feeding regime was kept as constant as possible, within the limits imposed by changing milk yield. The same cows, all of which had calved within 4 weeks of each other, were used throughout the lactation. The data reported thus represent changes in milk yield and composition which are due to changes in biochemical and physiological activity during the lactation. In this respect they differ from the other data presented in the literature, although

statistical methods have been used to eliminate the effects of other factors in at least one investigation.

Rumen samples were taken at two-weekly intervals throughout the main lactation period primarily as a check on the extent to which the attempt to eliminate dietary influences on lactation had succeeded. The results are the first obtained with the particular method of fatty acid analysis used and are of interest in themselves as a contribution to the field of ruminant nutrition. They also represent a long term study of rumen contents on a constant diet and are therefore discussed in some detail.

REVIEW OF LITERATURE

BIOSYNTHESIS OF MILK FAT

It has been appreciated for a very long time that the intake of dietary fat is usually insufficient to act as the sole source of milk fat and that fatty acids must be synthesised within the animal body.[1] Hilditch and Thompson[2] fed cows on a diet containing certain characteristic unsaturated fatty acids which were in turn isolated from the milk fat showing a contribution to milk fat by dietary lipids. Aylward et al[3] gave support to this suggestion when they administered iodised fat to lactating cows. They showed that iodised fat was secreted in milk in amounts directly related to blood levels, which were increased by drenching. Glascock et al[4] administered tritium labelled stearic acid and tristearin to a lactating cow and goat and showed that up to 50% of the activity appeared in milk fat. The long chain acids were very active but not the lower. From their calculations the authors suggest that up to 25% of milk fat is derived from dietary fat.

Graham et al[5] measured arteriovenous differences in cholesterol, total fatty acids, sugar, inorganic and total phosphorus across the mammary gland of the lactating cow and concluded that sufficient lipid was taken up to account for milk fat production. Meigs et al[6] using the same technique suggested that blood phospholipid was the source of milk fat. Roodzant[7] correlated blood levels of phospholipid, cholesterol, neutral fat and total lipids with milk fat content. Phospholipid was most highly correlated and was regarded as the source of milk fat. Allen[8] measured blood fat content and

milk fat production but was unable to show any correlation. However, Maynard et al[9] showed that blood lipid level was correlated with milk fat yield. Both Lintzell[10] and Graham et al[5] using the arterio-venous difference technique concluded that blood glycerides and not phospholipids were the source of milk fat. This was supported by Aylward et al[3].

Achaya and Hilditch[11] examined the fatty acids of milk glycerides and from their calculations suggested that the lower fatty acids were derived from oleic acid by oxidation and reduction processes and this was again repeated by Hilditch.[12] The work of Glascock[4] already described indicated that fatty acids were absorbed from the blood by the mammary gland but there was no evidence that the lower acids were derived by degradation. Lossow and Chaikoff[13] injected tripalmitin-1-^{14}C and octanoate-1-^{14}C into the lactating rat and showed that long and short chain acids were taken into the milk without alteration in chain length. Glascock[14] supported this view. As early as 1938 Graham et al[15] showed the mammary glands of lactating goats to have respiratory quotients (RQ) greater than unity indicating fat synthesis from a carbohydrate source. Smith and Dastur[16] worked with fasted cows and found an increase in milk fat unsaturation, owing to an increase in oleic acid accompanied by a proportional drop in lower fatty acids. The authors interpreted these results as indicating a synthesis going to completion i.e. that fatty acids are synthesised from small carbon fragments. Reineke et al[17] measured the RQ of the mammary glands of fasted and non-fasted goats and found that although above unity for the non-fasted it fell below unity for the fasted animals. They concluded that synthesis

of milk fat from oxygen rich substrates was limited to the low molecular weight acids of the fat.

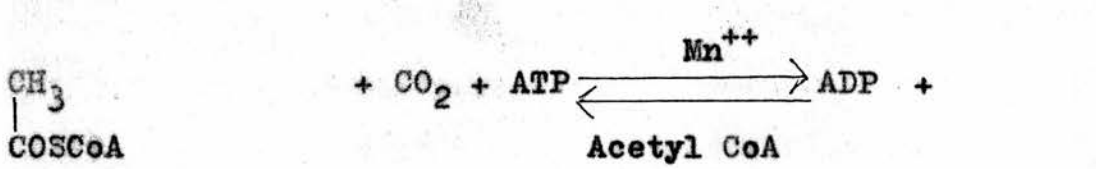
In a note to Nature[18] Folley and French described work with rat mammary gland slices and showed RQs greater than unity with glucose as substrate, indicating synthesis of oxygen poor substances from carbohydrate i.e. fat from glucose. Later[19] the work was extended to show that mammary gland slices from ruminants gave RQs of less than unity with glucose indicating no fat synthesis from glucose in these animals. The same authors[20] showed that slices from the mammary glands of pregnant animals gave RQs of less than unity with glucose but that the RQ rose after parturition to about 1.6 and remained at this level for the rest of the lactation. Although almost inert to glucose, mammary gland slices from ruminant animals were shown by Folley and French[21] to utilise acetate with an RQ greater than unity in contrast to those of non-ruminants which gave RQs below unity. Folley and French concluded that acetate appeared to be a primary source of fatty acids in ruminant mammary glands while glucose served a similar purpose in non-ruminants. McClymont[22] using the arterio-venous difference technique was able to show uptake of acetate by the lactating mammary gland of the cow and McClymont and Shaw[23] confirmed this by work on the half-udders of cows perfused with blood containing added acetic acid. Linzell[24] showed that acetate uptake by the mammary gland increased threefold on the onset of lactation. Hardwick et al[25] perfused the isolated goat udder with fluid containing labelled acetate and glucose and showed that 48% of the fatty acids of milk were derived from acetate, but only 0.3% of the milk lactose and 2% of the triglyceride glycerol was so derived. Popjak et al[26]

fractionated the milk fat of rabbits after injection with sodium acetate labelled at the carboxyl carbon. The soluble volatile fatty acids had the highest activity which was up to eighteen times that of the other acids. It was suggested that the volatile fatty acids were synthesised in the mammary gland from two carbon units including the carboxyl carbon of acetate. The same workers[27] injected (Carboxy-^{14}C) acetate into the jugular vein of the goat and measured the activity of respired carbon dioxide and various fatty acid fractions of the milk fat. They showed a rapid utilisation of acetate for milk fat production the lower acids having a higher activity than the higher acids. The authors considered that their results gave no support for the degradative theory of the origin of the lower acids of milk fat. Popjak et al[28] examined the mode of formation of milk fatty acids from acetate in the lactating goat mammary gland. After fractionation into individual acids they concluded that acids up to and including palmitic acid were formed by stepwise elongation of short chain acids by addition of two carbon units derived from acetate. They suggested also that a considerable contribution was made to butyric acid formation by a four carbon compound probably beta-hydroxybutyric acid. Rogers and Kleiber[29] injected labelled metabolites into the cow intravenously and compared the specific activities of the various fat constituents. They showed that glucose and propionate were important sources of glycerol but not of fatty acids. Acetate was the most important fatty acid precursor but also made a small contribution to glycerol formation. Riis et al[30] infused cow plasma containing P^{32} and 14-C labelled lipids

into a lactating cow and measured the activity of various plasma and milk constituents and of respired carbon dioxide. They estimated that about 50 per cent of milk fatty acids were derived from the plasma lipids while the remainder were synthesised in the mammary gland. Barry et al[31] using the arteriovenous difference technique with goats showed that the fatty acids of milk derived from the blood lipids, originated in the chylomicrons and low density lipoproteins of the plasma. They also showed considerable arterio-venous differences in beta-hydroxybutyric acid but not in acetoacetic acid.

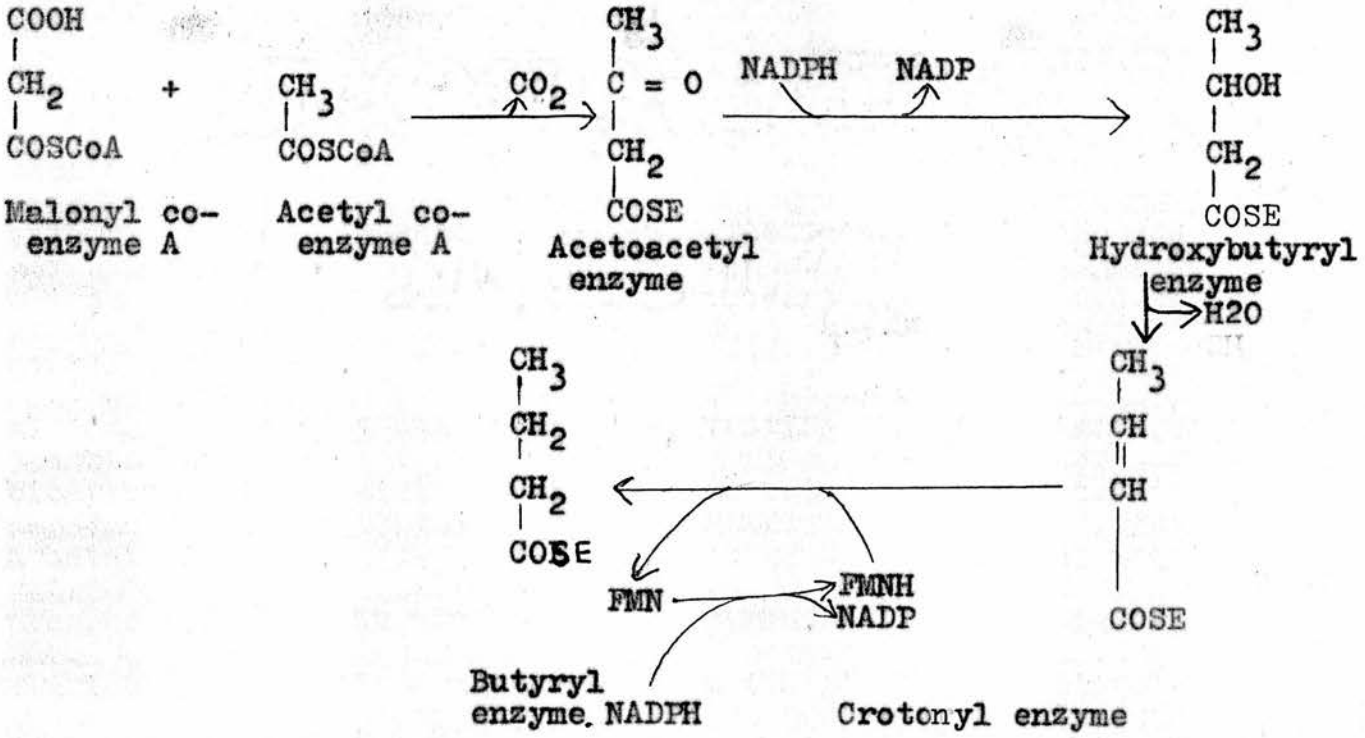
With the acceptance of acetate as an important precursor of milk fat a pathway for the synthesis was suggested and widely accepted and was in fact the reverse of the well known beta-oxidation pathway of fat degradation.[32] Popjak & Teitz[33.34] using udder tissue slices showed the synthesis to require coenzyme A, ATP and reduced coenzymes 1 and 2. Malonate stimulated the synthesis. Gibson et al[35.36] showed that palmitic acid formation in avian liver required bicarbonate ions which were not incorporated and suggested they had a catalytic role. Two enzyme systems were involved and required the presence of ATP, manganese ions, and TPNH. The mechanism was further elucidated by Wakil[37] who showed that the first system involved carboxylation of acetyl coenzyme A to an intermediate which he suggested might be malonyl coenzyme A. The second system was concerned with elongation of the carbon chain. Brady[38] showed the formation of fatty acids from malonyl coenzyme A in a pigeon liver system, while Formica and Brady[39] confirmed carboxylation of acetyl coenzyme A to a malonic

acid derivative. Wakil[40] concluded that the synthetic pathway was not the reverse of beta-oxidation and suggested that the first stage was carboxylation of acetyl coenzyme A to malonyl coenzyme A.



Acetyl coenzyme A — Malonyl Coenzyme A.

The malonyl coenzyme A would then react with further acetyl coenzyme A to give an acetoacetyl enzyme complex which underwent a series of reactions to give a butyryl enzyme complex.



This would then react with further malonyl coenzyme A to give a longer chain acyl derivative. The importance of this pathway in the cow was demonstrated by Gangully.[41] He used radioactive malonyl coenzyme A to show an enzyme system in the cow mammary gland which utilised it

to give fatty acids similar in nature and distribution to those of milk fat. Dils and Popjak[42] confirmed this with rat mammary gland tissue and showed that no synthesis took place when carboxylation was prevented with avidin. The malonyl coenzyme A pathway only proceeds to palmitic acid, and higher acids are produced by the mitochondrial system discussed by Wakil.[43] This involves a chain elongation of two carbon atoms by reaction of the acyl coenzyme A derivative with acetyl coenzyme A in the presence of ATP, reduced coenzymes and pyridoxal phosphate. The importance of this latter pathway is problematic in view of the evidence for the blood lipids as the major source of the high molecular weight acids.[4,31]

Unsaturated acids have been shown to be produced from saturated acids. Keeney et al[44] have shown ketostearate to be present in milk glycerides and suggest that such keto-acids may be intermediates in the transformation of saturated to unsaturated acids. A more likely mode of formation would appear to be dehydrogenation of the saturated acid which has been shown to take place in the udder by Lauryssens et al.[45]

Propionate has been shown by several workers to make only a very minor contribution to fat synthesis. Lauryssens et al[46] perfused an isolated udder with (1-^{14})C propionate and showed activity in the udder fatty acids. James et al[47] in very similar studies showed the odd numbered acids to have a higher activity than the even numbered acids. Degradation of n-pentadecanoic acid gave products indicating condensation of propionate with acetate. Popjak et al[26] showed activity in odd numbered acids after injection with radioactive acetate and concluded that these were formed by condensation of acetate and

propionate residues. The activity of even numbered acids after administration of radioactive propionate was ascribed to oxidation of the third propionate carbon to acetate.

Kleiber et al[48] injected (1 - ^{14}C) and (2 - ^{14}C) butyrate intravenously into dairy cows and measured the activities of the milk constituents. They found those of lactose and protein to be higher than that of the fat. The glycogenic rather than lipogenic nature of butyrate was confirmed by Lauryssens et al[49] who perfused half-udders with radioactive butyrate. They suggested that butyrate is not directly utilised for fat synthesis but is first broken down to two carbon fragments which may be so utilised.

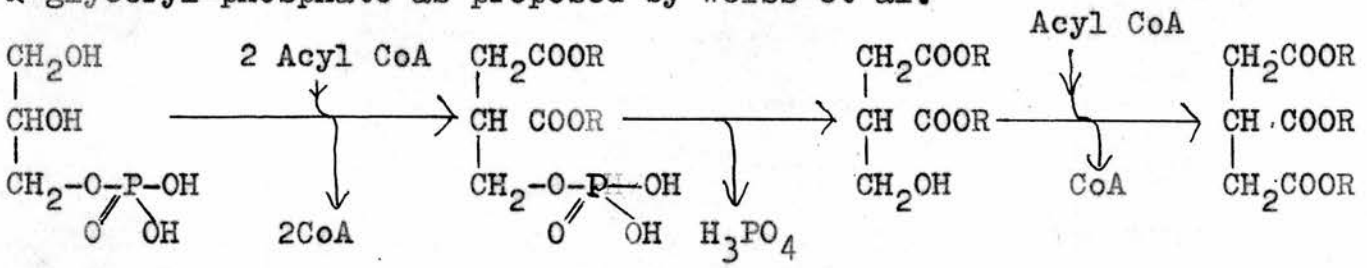
Shaw and Knodt[50] measured arterio-venous differences in beta-hydroxybutyrate across the mammary gland and showed a sufficient intake by the lactating gland of normal cows to account for the lower acids of milk fat. The non-lactating gland did not take up beta-hydroxybutyrate. Shaw[51] showed that the lactating glands of ketotic cows took up beta-hydroxybutyrate in greater amounts than those of normal cows and that it was used entirely as an energy source. Using the perfused lactating udder Shaw and Petersen[52] showed uptake of beta-hydroxybutyrate and noted that its use for formation of fatty acids could account for the RQ of greater than unity shown by normal udders using beta-hydroxybutyrate. Kumar et al[53] Lakshmanan et al[54] and Shaw and Lakshmanan[55] measured the transfer of radioactive beta-hydroxybutyrate from blood used to perfuse an isolated udder to the casein, lactose and fat of the milk produced. Their findings were similar to those obtained with acetate perfusions. Fat showed the

greatest activity and the activity of the individual fatty acids increased to caprylic and then decreased. Uptake of beta-hydroxybutyrate by the lactating gland appeared to be affected by the level of acetate in the perfusing blood, being lowest when acetate was high. More recently Barry et al[31] have confirmed an appreciable uptake of beta-hydroxybutyrate by the mammary gland of the lactating goat.

That the role of glucose in fat synthesis in ruminants was mainly as a source of glycerol and was insignificant in fatty acid synthesis has already been mentioned[29] and there is support for this view in the work of Balmain et al[56] on the incorporation of glucose by mammary gland slices. Work on isolated perfused cow udders by Peeters et al[57] and with goat udders by Hardwick et al[25] confirmed that fatty acid synthesis from glucose in the ruminant mammary gland was negligible. Folley and French[21] demonstrated the stimulating effect of glucose upon acetate utilisation by ruminant mammary gland slices. They found the RQ with acetate and glucose to be higher than with acetate alone. Balmain et al[56.58] showed greater incorporation of acetate carbon into fatty acids in the presence of glucose. This effect of glucose may be explained in terms of its specific role as a source of NADPH for fatty acid synthesis and as an energy source in sparing acetate for fat synthesis. It is interesting in the light of present knowledge to note the prediction of Folly and McNaught[59] that "TPNH may yet prove to be involved in lipogenesis in the mammary gland", and of Folley[60] that "the mammary gland may be able to utilise glucose by some pathway other than the

glycolytic".

The fatty acids absorbed by the mammary gland from the blood, along with those synthesised by the gland itself, are secreted in the milk as glycerides. Glyceride formation may take place from ∝-glyceryl-phosphate as proposed by Weiss et al.[61]



The ∝-glyceryl phosphate could be obtained from glucose and this agrees with the view of Popjak that milk fat glycerol was derived from glucose in the mammary gland. It may also be obtained from glycerol in the presence of ATP and glycerokinase. Barry et al[31] gave evidence for retention of glycerol by the lactating mammary gland. Dils and Clark[62] showed ∝-glyceryl phosphate to be the major source for glyceride formation although McBride and Korn[63] have shown mammary gland homogenates to be capable of utilising glycerol instead of glyceryl phosphate. Clark and Hubscher[64] have suggested that synthesis of glycerides in the intestinal mucosa may take place by direct esterification of monoglyceride but this does not appear to be of any significance in milk fat production.

VOLATILE FATTY ACIDS IN RUMEN LIQUOR

Tappeiner[65] showed considerable quantities of volatile fatty acids in the rumen of sheep. He considered that these were formed by cellulose breakdown and that acetic acid formed more than 50% of the total. The significance of these observations was not appreciated

and for many years the rumen was considered to be primarily a storage organ. A resurgence of interest occurred when McAnally and Phillipson,[66] Barcroft et al[67] and Phillipson and McAnally[68] showed large quantities of volatile fatty acids to be absorbed from the rumen into the blood. Many workers have analysed rumen liquors for individual volatile fatty acids and have shown acetic, propionic and butyric to be the major acids present with only small amounts of higher acids.[69.70.71.72.73.74] Other workers[75.76.77.78.79] have shown that in addition to the major acids rumen liquors may contain iso-butyric acid, iso-valeric acid, n-valeric acid, 2-methyl butyric acid and caproic acid. Brown and Shaw[74] showed that fasting lowered the total volatile fatty acids of the rumen and that the proportion of acetic acid was raised and that of propionic acid lowered. The authors examined both ketotic and normal cows but were unable to show any differences, unlike Schultz[72] who claimed higher acetic acid and lower propionic acid concentrations in the rumen liquors of ketotic cows.

THE RELATIONSHIP BETWEEN RUMEN VOLATILE FATTY ACIDS AND MILK FAT CONTENT

Tyznik and Allen[69] fed cows on diets containing as little as three pounds of hay and found a decrease in acetic acid and an increase in propionic acid in the rumen liquor compared to diets containing normal roughage levels. Butyric acid content remained constant. The low roughage depressed the milk fat content to between 1 and 2 per cent after two weeks but fat levels could be returned to normal by feeding up to one pound of sodium acetate per day. Ensor et al[70] fed various diets containing long and ground roughage, low roughage content and heated and unheated maize to milking cows. They

showed no difference in rumen volatile fatty acid pattern or in milk fat content between ground and long roughage diets. Addition of maize to the ground roughage diets caused a lowered milk fat content and the rumen liquors contained lowered acetic acid and raised propionic acid levels. Substitution of heated for unheated maize accentuated these changes but the effect was much reduced when about six pounds of long hay were included in the diet. Eusebio et al[71] confirmed the effect of heated maize in cow diets and quoted molar percentages of 33.9 for acetic acid and as high as 46.2 for propionic acid on all-concentrate diets. Balch et al[73] induced the production of low fat milk in fistulated cows by feeding diets low in hay and high in concentrates. The lowered fat was accompanied by lowered ruminal acetic acid and raised ruminal propionic acid levels. Butyric acid remained constant. Orth[80] linked diets high in soluble carbohydrate and low in crude fibre with ruminal liquors low in acetic acid and high in propionic acid, and the production of low fat milk, and Brown et al,[81] Shaw et al[82] and King and Hemken[83] confirmed this. Further evidence of the close association between the volatile fatty acids and milk production was provided by several workers who infused acids into the rumens of milking cows. Rook and Balch[84] infused acetic and propionic acids. Infusion of acetic acid increased yield and fat content while propionic acid increased milk yield and solids-not-fat content but decreased fat content. Storry and Rook[85] showed by similar infusions and blood analysis that acetic and butyric acids are ketogenic while propionic acid is glucogenic. Rook and Balch[86] confirmed the effect of acetic acid infusion on milk

production but found that propionic acid infusion reduced both yield and fat content of milk.

THE VOLATILE FATTY ACIDS IN BLOOD

Few workers have measured blood levels of volatile fatty acids on diets producing low fat milks. McLymont[87.88] showed differences in the volatile fatty acid content of peripheral blood on different diets but the proportions of acetic, propionic and butyric acids remained constant. Very small quantities of valeric, hexanoic, heptanoic and octanoic acids were shown to be present. Within a given feeding period there was a close relationship between ruminal volatile fatty acid content and blood acetic acid levels but no relationship between blood acetic acid content and milk fat content could be demonstrated. Van Soest and Allen[89] fed cows on diets containing restricted amounts of roughage and compared with normal diets, obtained low ruminal acetic acid to propionic acid ratios, although the concentration of both acids was increased. The composition of the blood volatile fatty acids was very similar on both normal and low roughage diets but blood acetic acid levels were slightly lower on the restricted roughage diets. Rook and Line[90] fed cows on very high energy diets and showed a drop in milk fat content, and an increase in yield and solids-not-fat. They showed blood volatile fatty acids to be higher on the high energy diets.

KETONE BODIES IN RUMEN CONTENTS

The rumen liquors of cows have been shown to contain ketones. Koffman[91] reported the presence of acetone in the rumen

liquors of cows showing signs of acetonaemia and linked high acetone with low pH. Boddie[92] drew attention to the presence of ketone bodies in rumen liquor but gave no figures and made no attempt to identify the actual substances involved. Iso-propanol was shown to be present in the rumen liquor of ketotic cows by Robertson et al.[93] Thin and Robertson[94] examined the rumen liquors of normal cows with a total ketone content of 0 - 10.25 mg/100 ml as acetone, and ketotic animals with up to 55.54 mg/100 ml. The authors claimed that beta-hydroxybutyric acid only, was present in normal liquors while acetone, acetoacetic acid and isopropanol were present in the rumen liquors of ketotic animals.

KETONE BODIES IN BLOOD

Thin and Robertson[94] showed that the level of ketone bodies in blood followed that in the rumen liquor but was very much higher. Again the blood of normal cows contained beta-hydroxybutyric acid only and the increased levels in ketotic animals were due to increases in the amounts of the other ketone bodies relative to the beta-hydroxybutyric acid. Bach and Hibbit[95] compared thirty ketotic and thirty six normal animals and showed levels of total ketones of 65.6 mg./100 ml. compared with 6.1 mg./100 ml. of acetone, in the blood. In the ketotic animals beta-hydroxybutyrate formed about one third of the total. The authors noted that ketone body levels were high in early lactation. Shaw[96] showed large increases in the level of ketone bodies in blood to 70 mg./100 ml. of acetone within one to three days post-partum. The author noted an increased uptake of beta-hydroxybutyric acid by the mammary glands of ketotic cows. Production of milk was adversely affected in cows showing high blood

ketone levels. Other workers[97.98.99.100.101] have shown high blood ketone levels as late as three months post-partum and Aafjes[100] showed that blood acetate levels increased with increase in blood ketone levels up to 5m.mole/l. Van Soest et al[102] fed dairy cows on low roughage diets and produced a drop in fat content from 4.62 per cent to 2.14 per cent and blood ketone level was reduced from 4.62 mg./100 ml. to 2.26 mg./100 ml. This was later confirmed by Van Soest and Allen.[89] Knodt[103] fed glucose to milking cows and brought about a fat depression accompanied by a reduction in blood ketone bodies. A seasonal variation in blood ketone level was demonstrated by Van Soest et al.[104] Levels up to 28 mg./100 ml. of acetone were unaccompanied by clinical signs of ketosis. A correlation between fat metabolism and stress was postulated. Knodt et al[105] examined the effect of various feeding regimes on blood ketone levels. They showed that silage fed animals had higher levels than concentrate fed animals which had higher levels than pasture fed animals. The high ketone levels in silage fed animals resulted mainly from an increase in beta-hydroxybutyric acid.

EFFECT OF LACTATION ON MILK YIELD AND COMPOSITION

Crowther[106] reviewed work to date and stated that milk quality was lowest in the second and third month of lactation. Eckles and Shaw[107] showed a decline in yield from a maximum value at thirty to eighty days but mostly between thirty and fifty days. Turner et al[108] working with Holsteins showed that maximum yield occurred later for animals with higher maxima. Tocher[109] in an examination of six hundred samples of milk from individual Ayrshire cows found fat content to be maximal between fourteen to sixteen weeks and solids-not-fat content at seventeen to twenty one weeks. Drakeley[110] from a study

of over 3,000 milk samples put the period of lowest quality much earlier at about forty days with maximum yield at about forty-five days. Several workers have shown very similar changes in milk composition during lactation.[111.112.113] Fat and solids-not-fat contents were high immediately post-partum and fell to minimal values at about forty days post-partum and there was then a gradual rise until about two hundred and fifty days when the rise became sharper to the end of the lactation. Johnson et al[114] working under American conditions showed that the fat content minimal period varied with breed, being the third month for Holsteins and the second month for Jerseys. Bartlett[111] showed maximum yield at forty days post-partum while Cannon et al[115] showed a decline in yield from month to month in the lactation. Waite et al[116] in a study of monthly milk samples from 3,000 pedigree Ayrshire cows confirmed that maximum yield occurred at about the forty-fifth day post-partum. Poorest quality milk was produced between forty five and seventy five days with a gradual improvement to two hundred days and a sharp rise thereafter.

EFFECT OF LACTATION ON CERTAIN BLOOD CONSTITUENTS

Several workers have examined the effect of parturition on the level of various metabolites in the blood. Van Soest and Blosser[117] analysed blood samples from dairy cows taken thirty, twenty and ten days and sixty and thirty hours, prepartum and thirty and sixty hours and ten, twenty and thirty days post-partum. They showed increased blood glucose levels in parturient cows and slightly lowered calcium and phosphorous levels. Haematocrit was higher at parturition

and then declined. Merril and Smith[118] in a similar experiment confirmed the high blood glucose level in newly calved cows but showed that this soon declined to normal. Wright et al[119] working with sheep showed that plasma globulin levels fell immediately prior to parturition while blood glucose fell to a minimum at maximum yield. Blood fat was high during pregnancy and fell during lactation. Knodt et al[120] sampled the blood of eleven cows monthly for twelve months. They showed a rise in blood ketones to a maximum value at ninety days post-partum followed by a gradual decline. Cessation of milking had no significant effect on blood ketone levels. The authors also examined the blood of 5 cows three days prior to and four days following calving. There was an apparent decline in blood ketones in the four days post-partum but this was non-significant. The composition of the ketone fraction remained constant throughout with a ratio of about 59 per cent beta-hydroxybutyric acid to 41 per cent acetone plus acetoacetic acid. Allen[8] from a study of fifty three complete lactations found low blood fat immediately before and after parturition. The level rose to the fourth or fifth month post-partum and then declined. He was unable to correlate blood fat levels with milk fat production. Maynard et al[9] took blood samples from four cows from immediately pre-partum to the end of their lactation. They showed a rise in blood lipids to 40 days post-partum and a decline to drying-off. There was no relationship between blood lipid and milk fat content but there was between blood lipids and yield of milk and fat.

EXPERIMENTAL METHODS

Three separate investigations were carried out in order to cover all phases of the lactation period from initiation to cessation of milking. The first covered that between calving and drying-off, the second the period during which calving took place, and the third, the period during which drying-off occurred.

SAMPLING

Blood

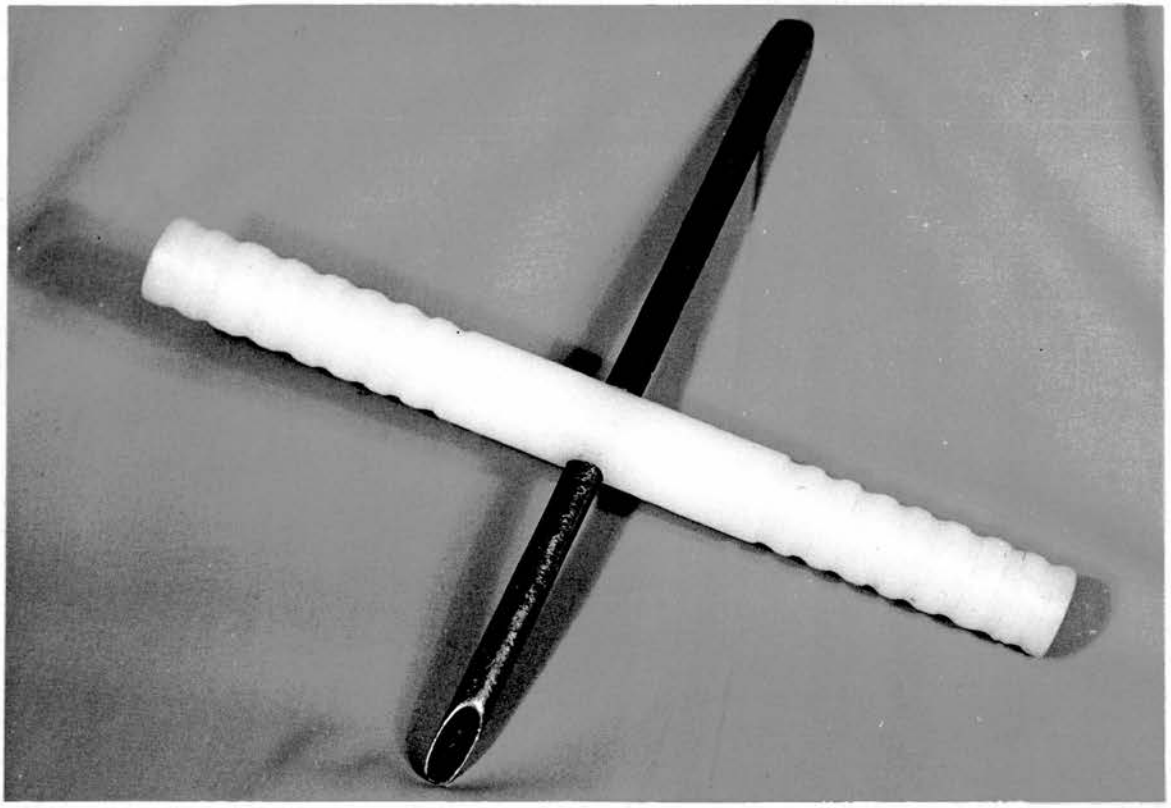
Blood samples were drawn from the jugular vein with as little upset to the cow as possible. Rook and Line[90] showed that there was a variation in blood composition in the post-feeding period and that the maximum concentration of volatile fatty acids occurred three to four hours after feeding. Knodt et al[105] showed maximum blood levels of ketone bodies at three hours after feeding. Samples were drawn at a specified three hours after the morning concentrate feed so that results would be comparable; also, at this period of maximal concentration, any differences would be accentuated. Heparin was used as anticoagulant.[121] pH was determined as soon as practicable using a Pye 78 pH meter with a Pye Ingold Combined Electrode at 20°C. The samples were then stored at 0-4°C to await analysis.

Rumen Contents

Samples of rumen contents were drawn immediately after taking the blood samples. Annison[78] and McLymont[88] showed peak ruminal volatile fatty acid concentration at three to four hours after feeding. Gray et al[76] and Bath and Rook[122] showed that different proportions of individual fatty acids were present in rumen contents at different times after feeding with propionic acid increasing and acetic acid decreasing

Fig. 1

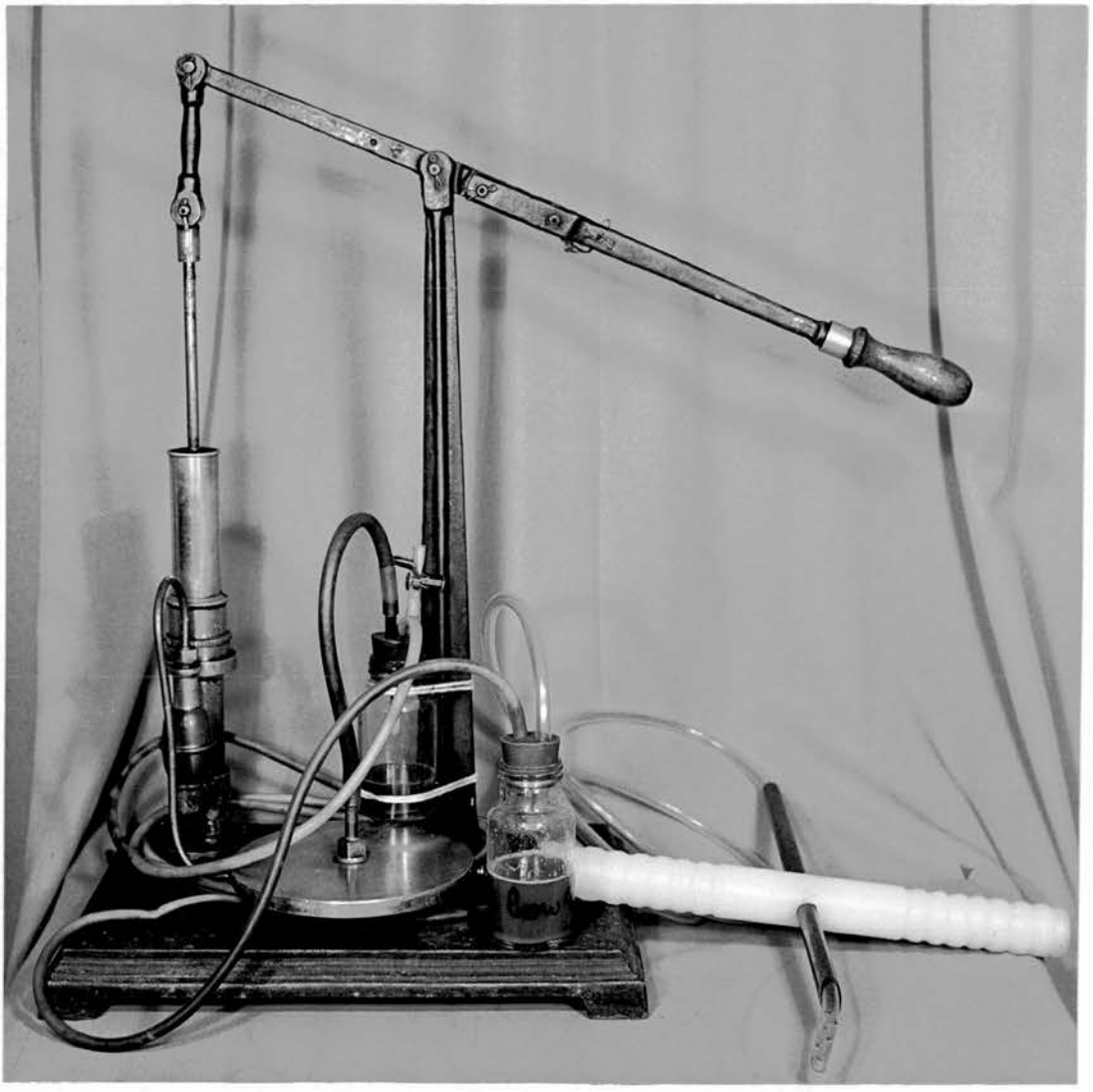
Mouth Gag for Sampling Rumen Contents



for the first few hours. Despite this it was considered that a sample of rumen contents taken at the time of maximal volatile fatty acid concentration was satisfactory to characterise the type of rumen fermentation taking place. A stomach tube and vacuum pump were used for sampling. The tube which was made of clear polythene was 2.25 m. in length with OD 11 mm. and ID 8 mm. The stomach end of the tube was cut obliquely and the last 6 cm. was perforated by ten holes 4 mm. in diameter. The greatest danger of the technique was that the tube could be passed into the lungs instead of the rumen. In order to guard against this a gag was used to carry the tube back over the tongue and clear of the opening of the trachea. The gag consisted of a 40 cm. cylindrical polythene rod, 4 cm. in diameter (Fig. 1). Both ends of the rod were corrugated for a length of 13 cm. to facilitate holding while in the mouth. A copper pipe (OD 1.6 cm; ID 1.3 cm) was passed through the middle of the rod. The pipe, cut obliquely at the end, was 40 cm. long and was adjusted so that about 15 cm. protruded into the mouth. For sampling the gag was held in the angle of the jaw so that the copper pipe lay over the back of the tongue with the cut side uppermost. The sampling tube was then passed through the copper tube into the oesophagus and so into the rumen and the sample withdrawn. On some occasions there was some contamination of the sample with saliva but this was easily detected and the sample discarded before withdrawing further rumen contents. The whole system is illustrated in Fig. 2. The samples of rumen contents were cooled and used for a pH determination as soon as practicable. They were then strained through four layers of cheese

Fig. 2

Apparatus for Sampling Rumen Contents



cloth and 1 ml. saturated mercuric choride solution was added per 50 ml. of liquor. This was then stored in a deep freeze to await analysis.

Milk

Cows were bucket milked and samples were taken from the well mixed contents of the bucket at the a.m. and p.m. milkings on the day of sampling. Samples were taken on three days of the week in which rumen and blood samples were taken and the six samples bulked according to yield to give a weekly-composite sample.

ANALYTICAL METHODS

Deproteinisation of Blood

Deproteinisation was carried out according to the method described by Thin and Robertson.[123] To 100 ml. of blood was added 200 ml. distilled water, 300 ml. hydrated zinc sulphate solution (2.5 per cent W/V) and the equivalent of 300 ml. of 0.15 N barium hydroxide solution. The whole was stirred and centrifuged for ten minutes at 2000 r.p.m. The supernatant liquid was decanted and used for analysis.

Determination of Acetone + Acetoacetic Acid in Blood

The method was that used by Reid.[124] 5 ml. of deproteinised blood was acidified with 8 ml. of 7N sulphuric acid and distilled. The distillate contained free acetone and acetone produced from acetoacetic acid. The acetone was estimated by reaction with alkaline ethanolic salicylaldehyde to give a yellow colour. The intensity of colour was read in a Unicam SP600 spectrophotometer at 530 mμ and the concentration of acetone read off a curve constructed using

standard acetone solutions.

Determination of Beta-hydroxybutyric Acid in Blood

To the residue of the distillation of deproteinised blood with sulphuric acid in the previous determination were added 5 ml. of potassium dichromate solution (0.2 per cent W/V) and the distillation continued. The distillate contains acetone produced by oxidation of beta-hydroxybutyric acid. The concentration was determined as previously described.

Determination of Total Volatile Fatty Acids in Blood

10 ml. of deproteinised blood were acidified with 2 ml. of 2N sulphuric acid and steam distilled in a Markham Still.[125] 1 ml. of 2 per cent octan-2-ol was added to prevent frothing as suggested by Scarisbrick[126] 70 and 30 ml. portions of distillate were collected, aerated with carbon dioxide free air for ten minutes and titrated with 0.01 N Sodium Hydroxide solution. Aeration was continued throughout the titration. The 30 ml. value was used as a blank. Recoveries of standard acid solutions put through the precipitation and distillation were of the order of 96 to 100.5 per cent.

Determination of Individual Volatile Fatty Acids in Blood

180 ml. of deproteinised blood were made alkaline with 4 N potassium hydroxide solution and evaporated to small bulk on a steam bath. Evaporation was carried to completion in a rotary film evaporator. The residue was dissolved in 2 ml. of 30 per cent orthophosphoric acid in an ice-salt bath. The free fatty acids were extracted into 2.5 ml. of diethyl ether (AR) the whole being maintained in the freezing bath. The acids were determined by gas-liquid chromatography of a 2µl aliquot of the ether solution using a Perkin Elmer F11 Gas Chromatograph. Operating conditions were as

FIG.3.

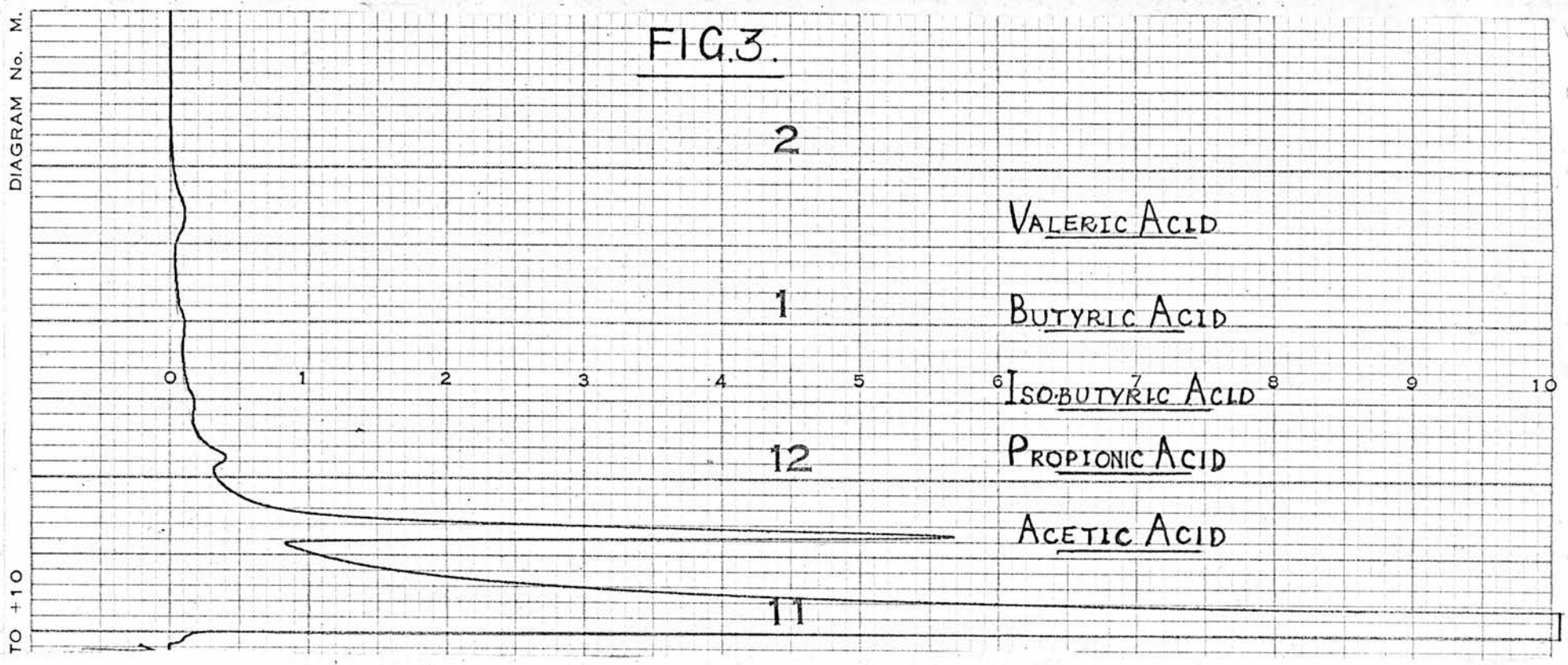


DIAGRAM No. M.
TO +10
2
1
12
11
0
1
2
3
4
5
6
7
8
9
10
VALERIC ACID
BUTYRIC ACID
ISO-BUTYRIC ACID
PROPIONIC ACID
ACETIC ACID

follows:-

Column	2m x $\frac{1}{8}$" OD stainless steel
Column packing	Trimer acid + dinonylnaphthalenedisulphonic acid on chromosorb W (4.5 : 0.5 : 95)[127]
Column temperature	110°C
Injection block temperature	180°C
Detector	FID
Sensitivity	20 x 1
Chart speed	15" per hour
Nitrogen pressure	12 p.s.i.
Compressed air pressure	22 p.s.i.
Hydrogen pressure	15 p.s.i.

The detector response was recorded on a Kent Mark 3, 0 - 2.5 mV recorder with a one second response. Peak heights were measured. A typical recorder trace is shown in Fig. 3. Individual acids were identified by retention times compared with those of pure standard acids (BDH). The amounts of acids were calculated by relating peak height to those of standard amounts of pure acids in ether solution. The standard solutions were subjected to exactly the same treatment as the whole blood to include the effect of individual acid response to the detector and differences in partitioning of the acids between ether and aqueous solution. Recoveries by this procedure were of the order of 99 to 100 per cent.

Determination of Acetone + Acetoacetic Acid and Beta-hydroxybutyric Acid in Rumen Liquor.

To 20 ml. of strained rumen contents were added 40 ml. of water, 60 ml. hydrated zinc sulphate solution (2.5 per cent) and the equivalent of 60 ml. of 0.15N barium hydroxide solution. The whole was stirred and filtered through a Whatman No. 12 fluted filter paper. 5 ml. of the filtrate was used for the determination of acetone plus acetoacetic acid and of beta-hydroxybutyric acid as previously described.

Determination of Total Volatile Fatty Acids in Rumen Liquor

10 ml. of strained rumen contents were mixed with 10 ml. of a saturated solution of magnesium sulphate in 2.5 per cent sulphuric acid solution and allowed to stand overnight at 0 to 4°C.[121] The precipitate was filtered through a Whatman No. 40 filter paper and 2 ml. of the filtrate used for the determination of total volatile fatty acids by steam distillation as previously described.

Determination of Individual Volatile Fatty Acids in Rumen Liquor

10 ml. of strained rumen contents plus 2 ml. 5N sulphuric acid containing 25 per cent metaphosphoric acid were allowed to stand for two hours at 0 - 4°C and filtered through a Whatman No. 40 filter paper. 6 ml. of filtrate were made alkaline to phenolphthalein with 4N potassium hydroxide solution in an ice bath. The solution was then evaporated to dryness in a rotary film evaporator. The residue was cooled in an ice bath, 4 ml. of a 30 per cent orthophosphoric acid solution were added and the whole shaken thoroughly while still in the ice bath, 5 ml. of ether were added, the whole again shaken thoroughly and 2 µl of the ether solution used for gas-liquid

FIG. 4.

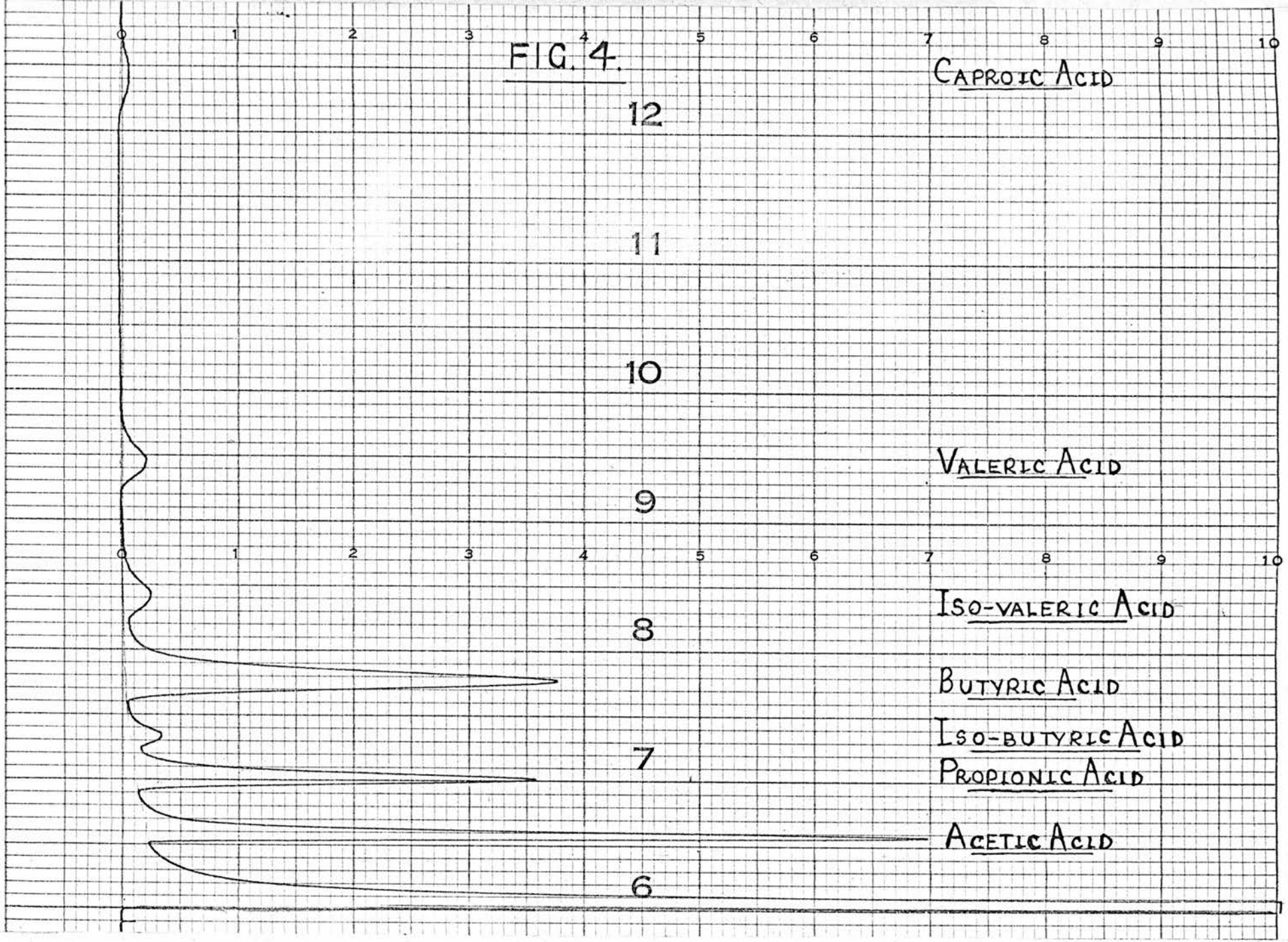


DIAGRAM No. M.6215

RANGE: -1 TO +10

chromatography. A typical recorder trace is shown in Fig. 4.

The technique was basically as previously described except that the instrument was used at the lower sensitivity of 1 x 10^2. Relative peak areas were measured with a Kent solid state integrating converting system and recorded by an ENM print out counter. Concentrations were calculated by relating recorded peak areas to those obtained with standard acid solutions put through the complete procedure.

Determination of Total Solids in Milk

Total solids content was determined by a gravimetric procedure in which 1 ml. of milk was weighed and evaporated to dryness on a hot plate at 136°C.[128] The residue was weighed after a further period of two hours in a fan oven at 100°C.

Determination of Fat Content of Milk

The method used was that given in British Standards (1955) BS 696.

Determination of the Lactose Content of Milk

Lactose was determined by the method of Hinton and Macara[129] using a modified zinc ferrocyanide coagulation technique.

Determination of the Crude Protein Content of Milk

Crude protein was determined by a semi-microkjeldahl technique using a selenium and potassium sulphate catalyst mixture.

Determination of Chloride Content of Milk

Chloride content was determined by a modification of the wet oxidation method of Davies.[130]

EXPERIMENT I

Investigation of the Period from Parturition to Cessation of Milking

EXPERIMENTAL

Animals

Six Ayrshire cows were used for the experiment. Details are given in Table 1.

Table 1

Details of Cows used in Experiment I

Cow	Age Years	Lactation	Live Weight lb.
1	6	4	992
2	6	4	1036
3	4	2	1030
4	7	4	1063
5	4	2	1025
6	6	4	1050

Feeding and Management

The cows were housed in an ordinary byre from about two months before calving to drying-off. All animals were given a basic ration of sixteen pounds of hay throughout the experiment. The composition and nutritive value of the hay are given in Table 2.

Table 2

	per cent
Dry Matter	82.0
Crude Protein	5.8
Crude Fibre	25.0
Calcium	0.33
Phosphorus	0.18
Magnesium	0.10
Estimated DCP	2.3
Estimated SE	31

Estimates of DCP and SE were made using the regression equations of Watson and Nash.[131] At about two months pre-partum, two

pounds of a concentrate mix were fed per day and this was increased until about eight pounds per day were being offered at parturition. The concentrate mix was made up of fourteen parts of barley, three parts of oats, one part of bran, one and a half parts of groundnut cake and one and a half parts of soyabean meal. A mineral mix consisting of seventy parts of steamed bone flour, twenty parts of feeding ground limestone and ten parts of salt was included at 3 per cent of the diet. The composition and nutritive value of the concentrate mix is given in Table 3.

Table 3

Composition and Nutritive Value of Concentrate used in Experiment I

	per cent
Dry Matter	86.6
Crude protein	14.2
Ether Extract	2.3
Crude Fibre	5.6
Nitrogen-free-extractives	58.6
Ash	5.9
Calcium	0.97
Phosphorus	0.74
Magnesium	0.15
Estimated DCP	12.5
Estimated SE	70

After calving no concentrate was offered for two to three days but hay was available at all times. The concentrate allowance was then built up rapidly to full feed at about two weeks post-partum. From this stage to peak yield the concentrates were fed at 110 per cent of the allowance based on yield at four pounds per gallon. Concentrates were given at 6.0 a.m. and 3.30 p.m. Hay was fed twice daily at 11.0 a.m. and 5.0 p.m. There were no refusals of concentrates, except for cow No. 6, to be discussed later, and the occasional hay

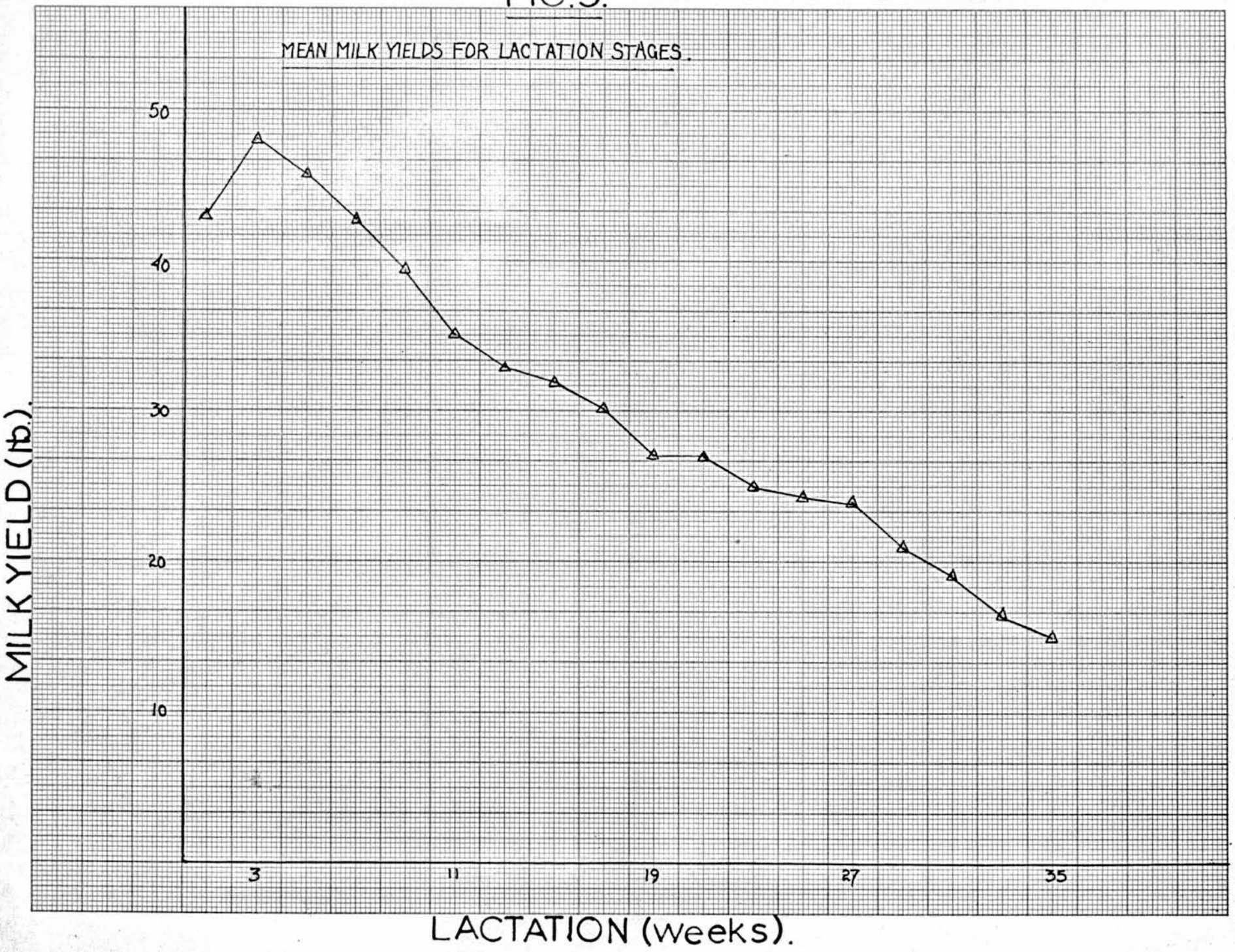
residues were small and nutritionally unimportant. Cows were bred at the second oestrus and were in calf from 8 weeks post-partum. Cow No. 6 was not bred at all.

Samples of blood and rumen contents were taken at seven days post-partum and at fourteen day intervals thereafter. Composite milk samples were taken during the same weeks as the rumen and blood samples except for the first week when one twenty four hour composite was taken on the seventh day. Milk yield was recorded at the milkings at which sampling was carried out. The strip cup was used at each milking as a check on udder health and as a further control, chloride determinations were carried out on each milk sample taken.

Cow No. 6 was destroyed after about three months and although results are presented in Appendix Tables 21, 22 and 23 these have not been included in any calculations or discussion of results. From the beginning of the experiment this animal lost condition and had little or no appetite. She lost a great deal of weight and her milk yield declined until she was practically dry by the eleventh week post-partum. At this time the milk showed a high fat content with a low solids-not-fat which was due to a fall in protein content. Rumen fluid samples showed a normal composition except for a low acetic acid, particularly marked in two samples for weeks seven and nine. The blood samples had very high levels of beta-hydroxybutyric acid throughout the period under test although acetone levels were normal.

The length of lactation varied, two cows being dried off after 35 weeks, two after 37 weeks and one persisting for 47 weeks. In some of the statistical calculations a 35 week lactation period has been adopted as this was the only period for which results for all five

FIG. 5.


MEAN MILK YIELDS FOR LACTATION STAGES.
50
40
30
20
10
MILK YIELD (lb.).
3
11
19
27
35
LACTATION (weeks).

cows were available and an examination of the data indicated relatively little change in the cows which milked after this.

RESULTS

Milk

The data collected on the yield and composition of milk are given in Appendix Tables 1, 5, 9, 13, 17 and 21.

Yield The milk yields of all cows show much the same picture with a rise in the early weeks of lactation followed by a decline which becomes more rapid as the drying-off stage is approached. Individual cows differ in level of yield, in the time taken to reach peak yield and the speed with which yield declines. An analysis of variance based on a thirty-five week lactation gave significant evidence of differences between cows and between stages of lactation. Mean figures for milk yield for the five cows at the various stages throughout lactation are shown in Fig. 5. Yield rises to a peak at three weeks and then falls to the end of lactation. Table 4 shows the mean yield at each stage of lactation as a percentage of that of the previous stage.

Table 4

Change of Milk Yield with Stage of Lactation

Stage of Lactation	2	3	4	5	6	7	8	9
Yield. per cent of previous stage	111.3	96.5	92.0	92.5	88.5	94.2	94.6	90.5
Stage of Lactation	10	11	12	13	14	15	16	17
Yield. per cent of previous stage	101.0	91.2	98.0	98.0	87.5	92.0	85.0	90.8

There is a period in mid-lactation when the change in yield is less marked than in early or late lactation.

FIG. 6.

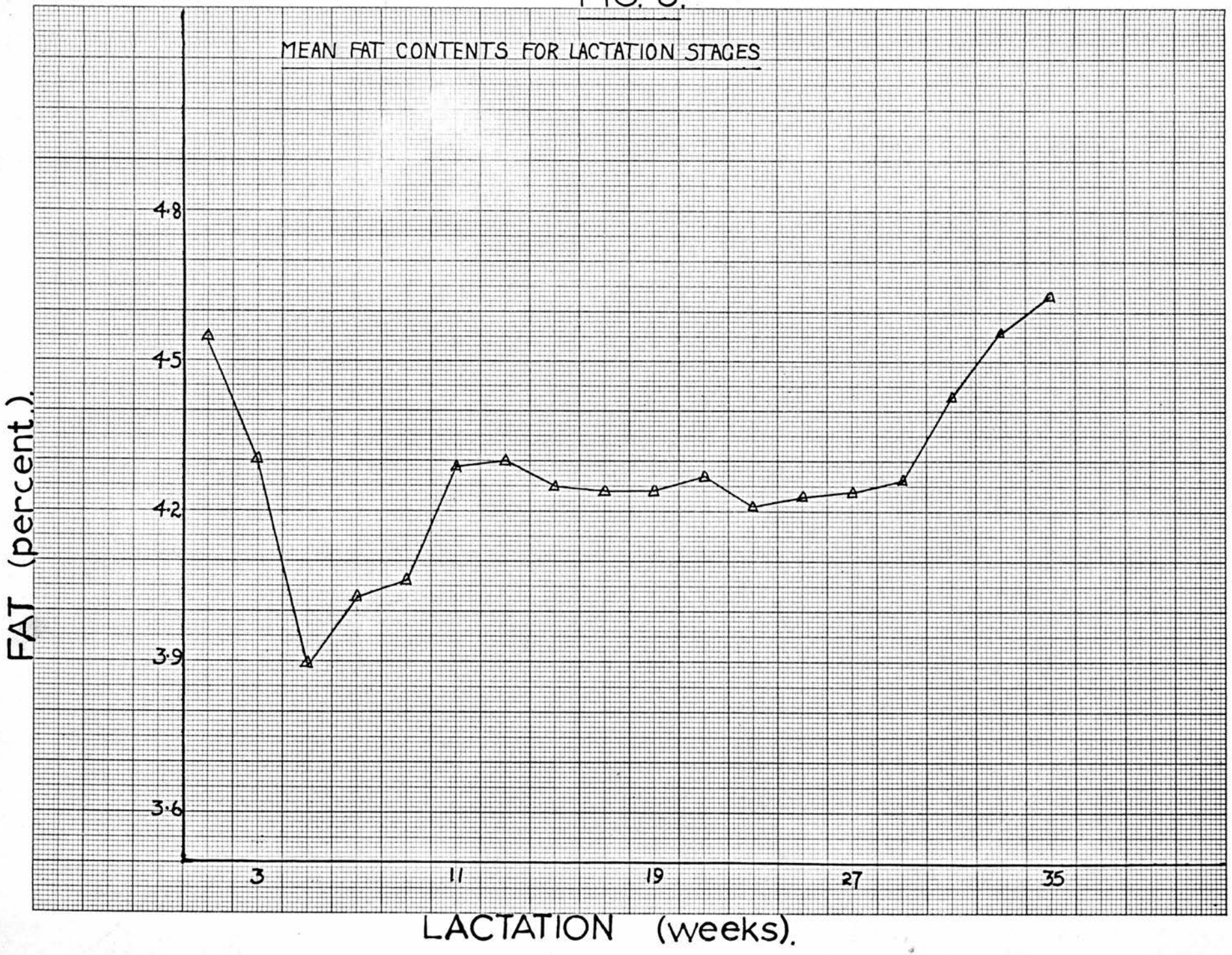
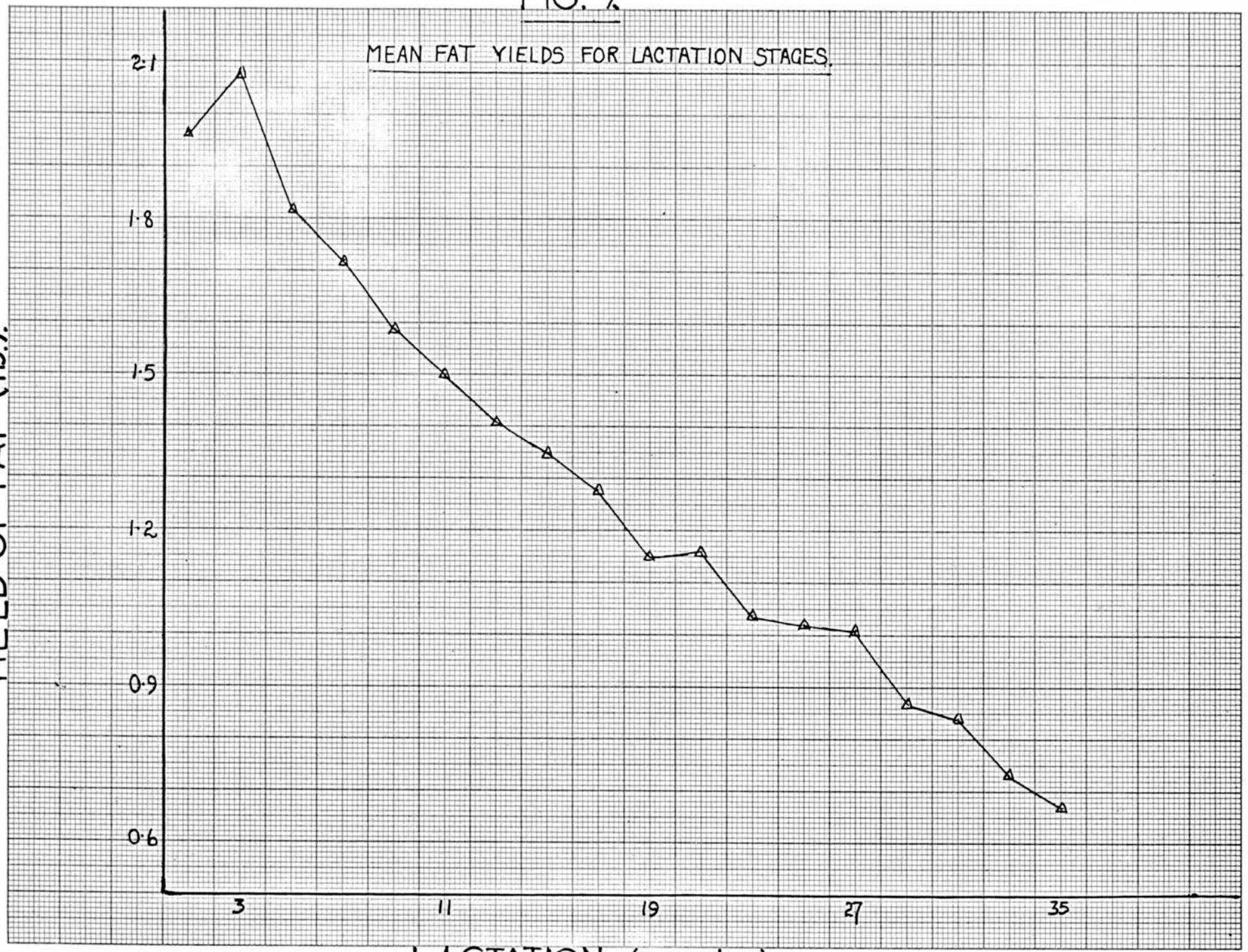

MEAN FAT CONTENTS FOR LACTATION STAGES
4·8
4·5
4·2
3·9
3·6
FAT (percent.)
3
11
19
27
35
LACTATION (weeks).

FIG. 7.


MEAN FAT YIELDS FOR LACTATION STAGES.
2·1
1·8
1·5
1·2
0·9
0·6
YIELD OF FAT (lb.).
3
11
19
27
35
LACTATION (weeks).

Fat Content The figures for the fat content of the milks of individual cows in the appendix tables show a fall in the first weeks of lactation a minimum being reached between the fifth and the ninth week post-partum. The shapes of the lactation curves for individual cows show differences. An analysis of variance on a thirty-five week lactation confirmed significant differences between cows and between stages of lactation ($P = 0.001$). Mean figures for the fat content of the milk of the five cows at different stages of lactation have been used to construct the graph shown in Fig. 6. Fat content is high in the first week but falls to a minimum at the fifth week post-partum and then rises sharply to the eleventh week after which it remains relatively constant to the twenty ninth week and then increases sharply once more.

Yields of fat have been calculated from the data for yield and fat content. An analysis of variance showed significant evidence ($P = 0.001$) for differences between cows and between stages of lactation. Mean values for the five cows at different lactation stages have been used to construct the graph shown in Fig. 7. The curve is similar to that for yield, having a peak at the third week. Subsequently the decline is sharper and is almost linear.

Solids-not-fat Content The solids-not-fat content of the milks of all cows is high at the beginning of lactation and falls to a minimum value in the first few weeks. There is a mid-lactation period of relatively constant composition before a final rise in late lactation. Individual cows show variation in level of solids-not-fat and the shape of the lactation curve e.g. minimum values occur from the fifth to the eleventh week post-partum. An analysis

FIG. 8.

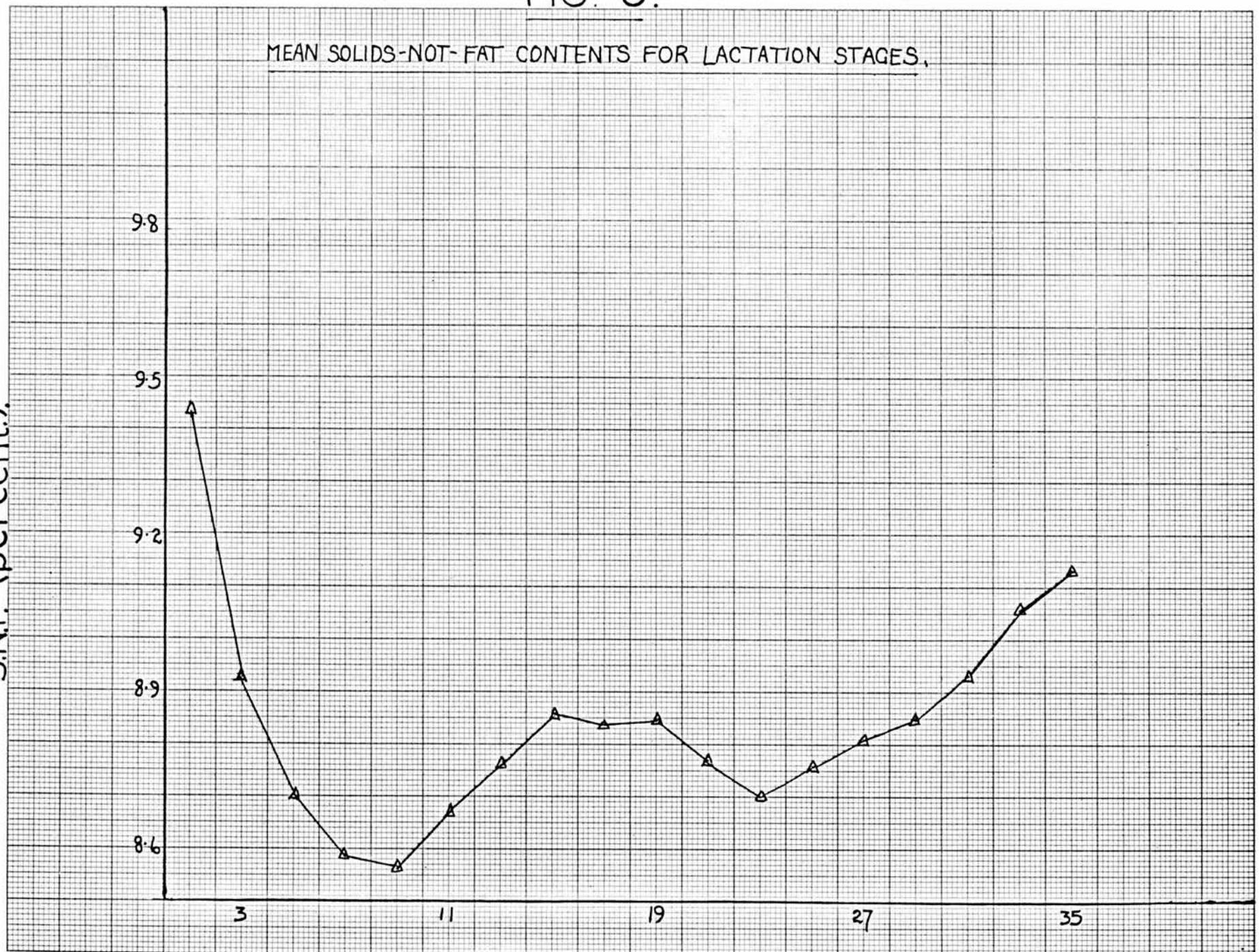

MEAN SOLIDS-NOT-FAT CONTENTS FOR LACTATION STAGES.
9·8
9·5
9·2
8·9
8·6
S.N.F. (percent.)
3
11
19
27
35
LACTATION (weeks).

FIG. 9.

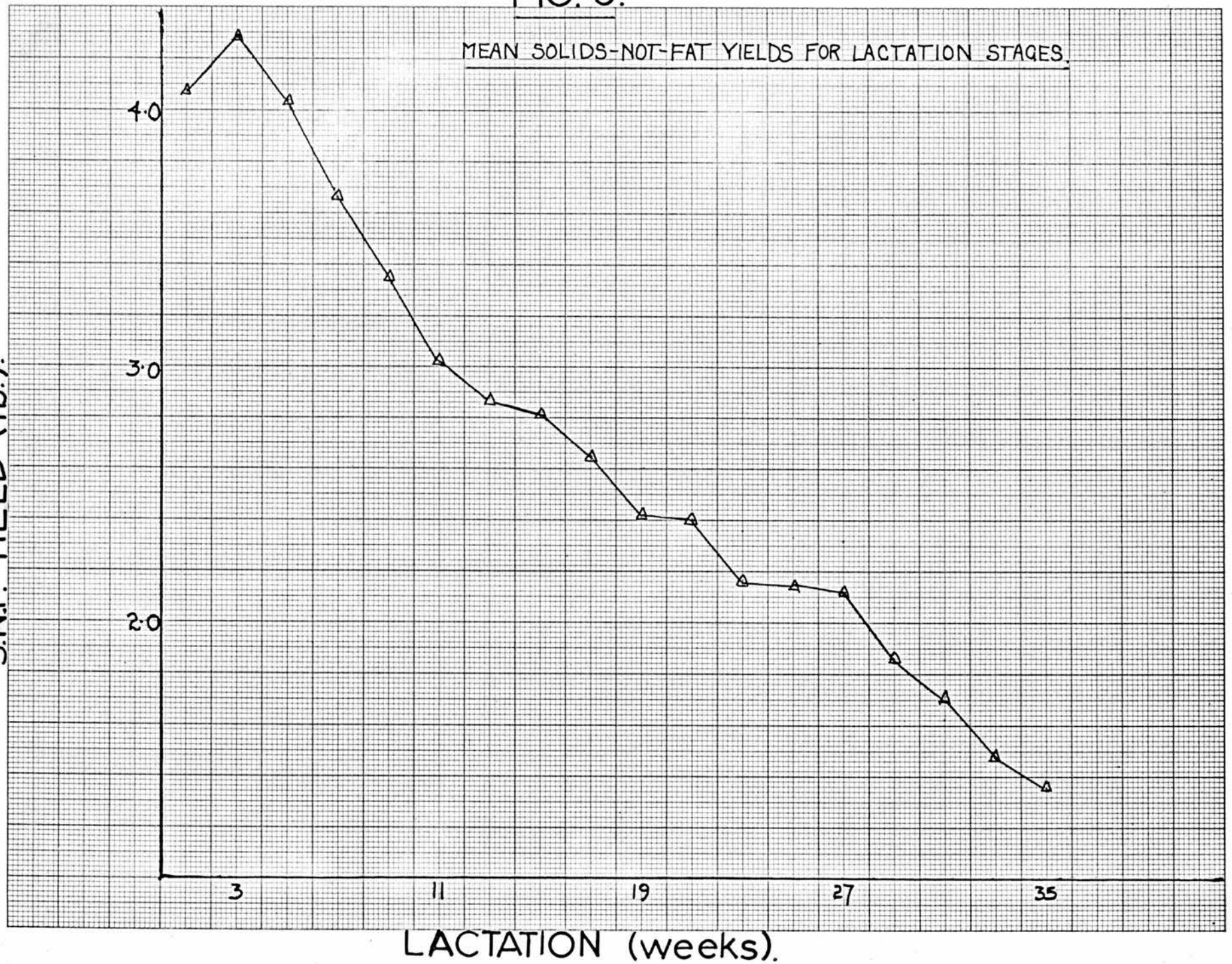

MEAN SOLIDS-NOT-FAT YIELDS FOR LACTATION STAGES.
S.N.F. YIELD (lb.).
4·0
3·0
2·0
3
11
19
27
35
LACTATION (weeks).

FIG. 10.

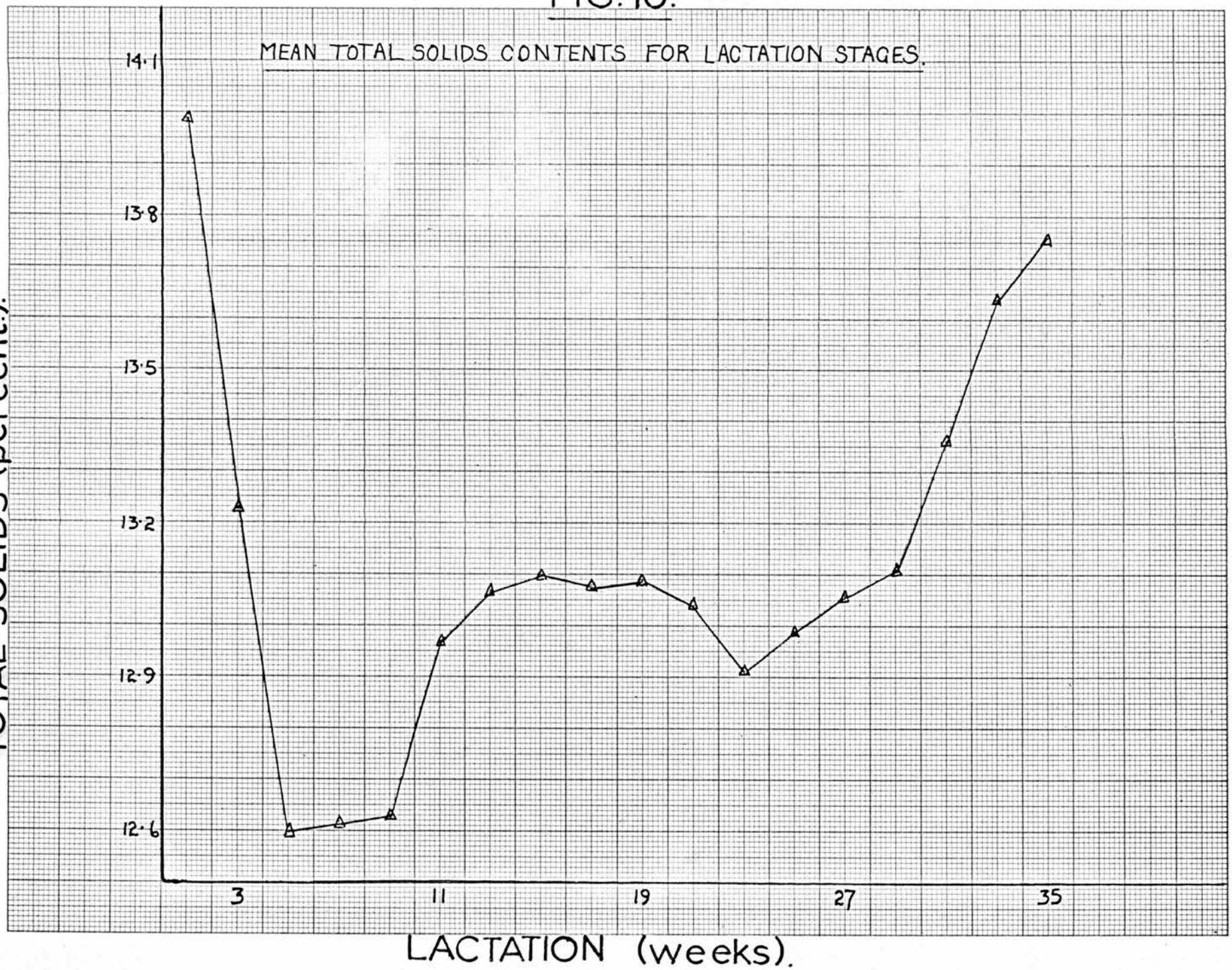
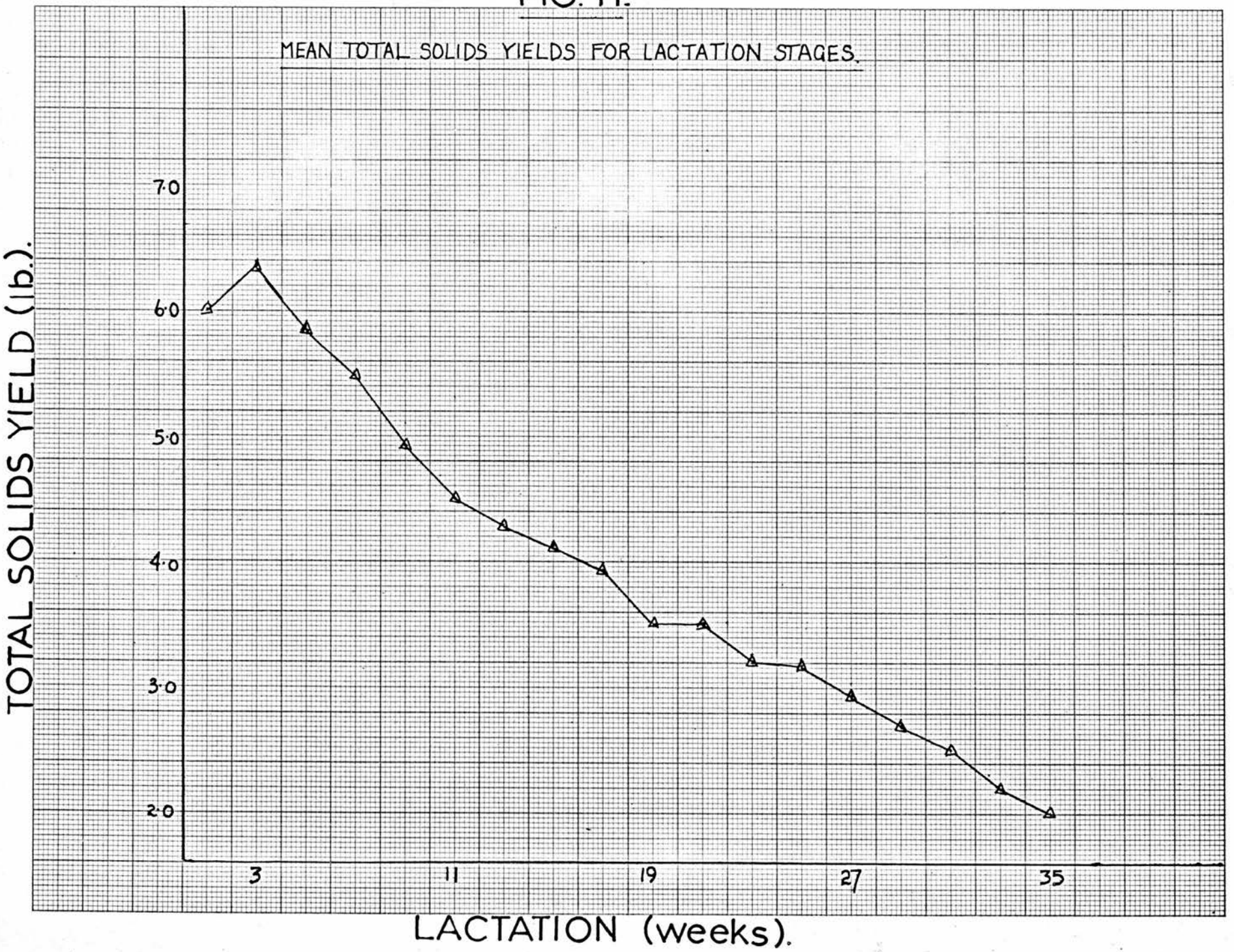

MEAN TOTAL SOLIDS CONTENTS FOR LACTATION STAGES.
14·1
13·8
13·5
13·2
12·9
12·6
TOTAL SOLIDS (percent.).
3
11
19
27
35
LACTATION (weeks).

FIG. 11.


MEAN TOTAL SOLIDS YIELDS FOR LACTATION STAGES.
TOTAL SOLIDS YIELD (lb.).
7·0
6·0
5·0
4·0
3·0
2·0
3
11
19
27
35
LACTATION (weeks).

of variance shows significant evidence of differences between cows and between stages of lactation. Mean values for the milks of the five cows are shown in Fig. 8. The shape of the curve resembles that for fat but the minimum occurs later and recovery is not so quick. The apparent secondary minimum occurring at the twenty third week post-partum is not significantly lower than the level of solids-not-fat at this stage of lactation.

Solids-not-fat production has been calculated and an analysis of variance confirmed significant differences between cows and between stages of lactation. Mean values for the five cows over the lactation are shown in Fig. 9. The curve is very similar to that for fat production.

Total Solids Content Total solids content is the sum of the fat content plus solids-not-fat content. Analysis of variance gave significant evidence for a difference between cows and between stages of lactation. Fig. 10 shows a lactation curve based on mean figures for the total solids content of the five cows. Total solids content is high at the beginning of lactation, falls to a minimum at thirty five days and remains low to the sixty third day. There is then a sharp rise to the seventy seventh day followed by a period of little change to the two hundredth day and a sharp rise thereafter. Owing to the fact that lactation changes in fat and solids-not-fat content are similar, total solids content shows a wider variation than either.

Total solids production (Fig. 11) shows a curve very similar tothose for fat and solids-not-fat but covering a wider range.

Lactose Content Unlike the constituents discussed so far

FIG. 12.

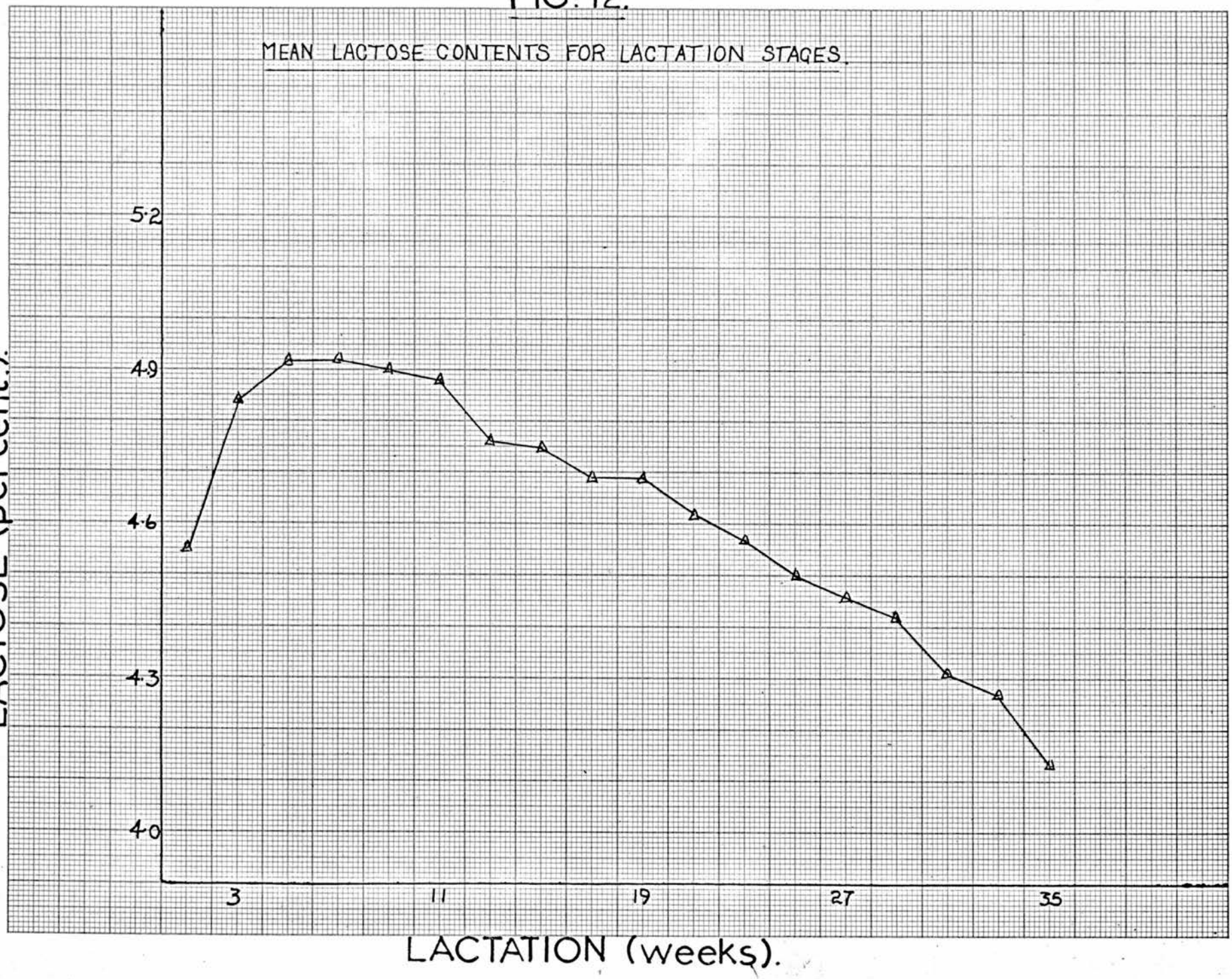

MEAN LACTOSE CONTENTS FOR LACTATION STAGES.
5·2
4·9
4·6
4·3
4·0
LACTOSE (percent.).
3
11
19
27
35
LACTATION (weeks).

FIG. 13.

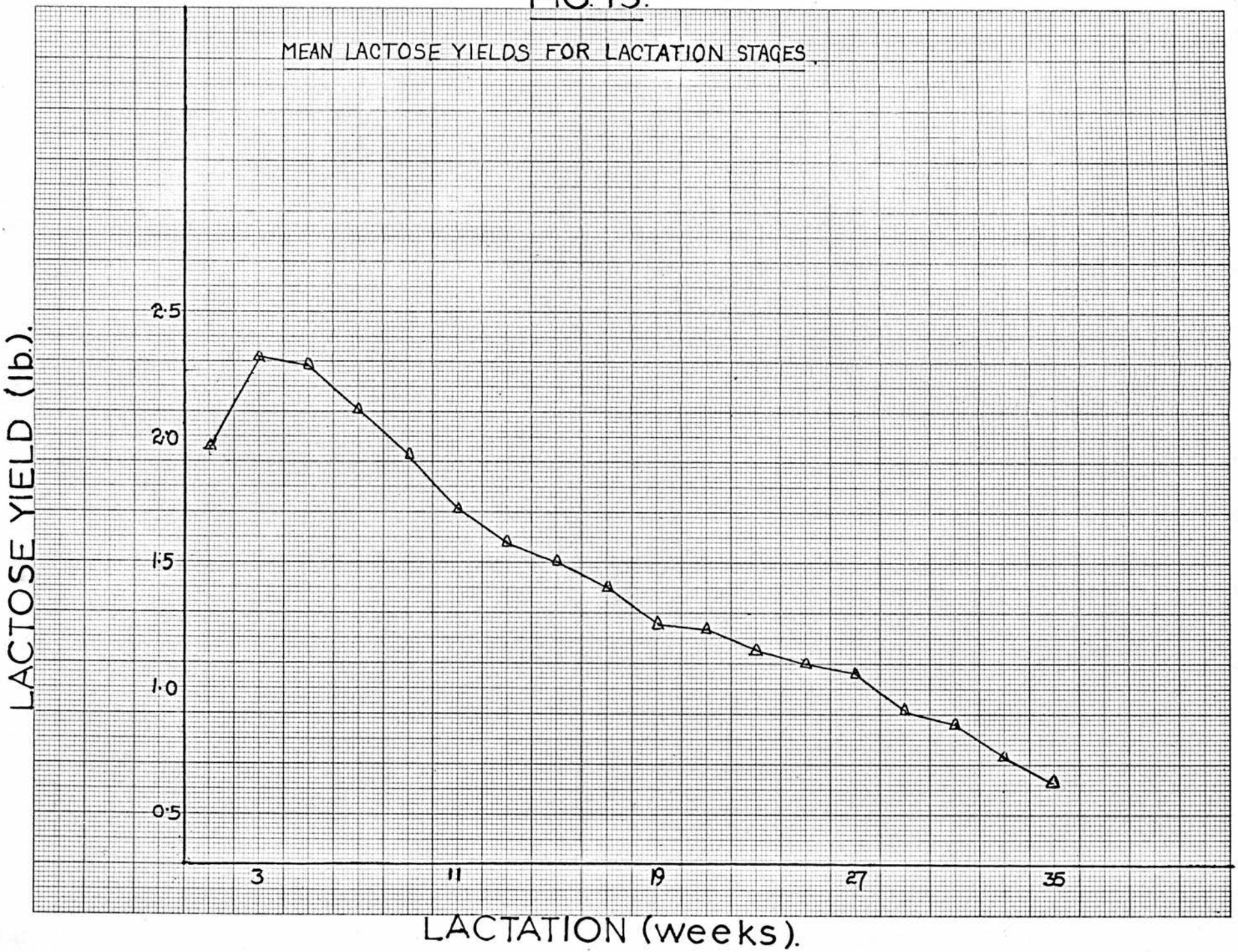
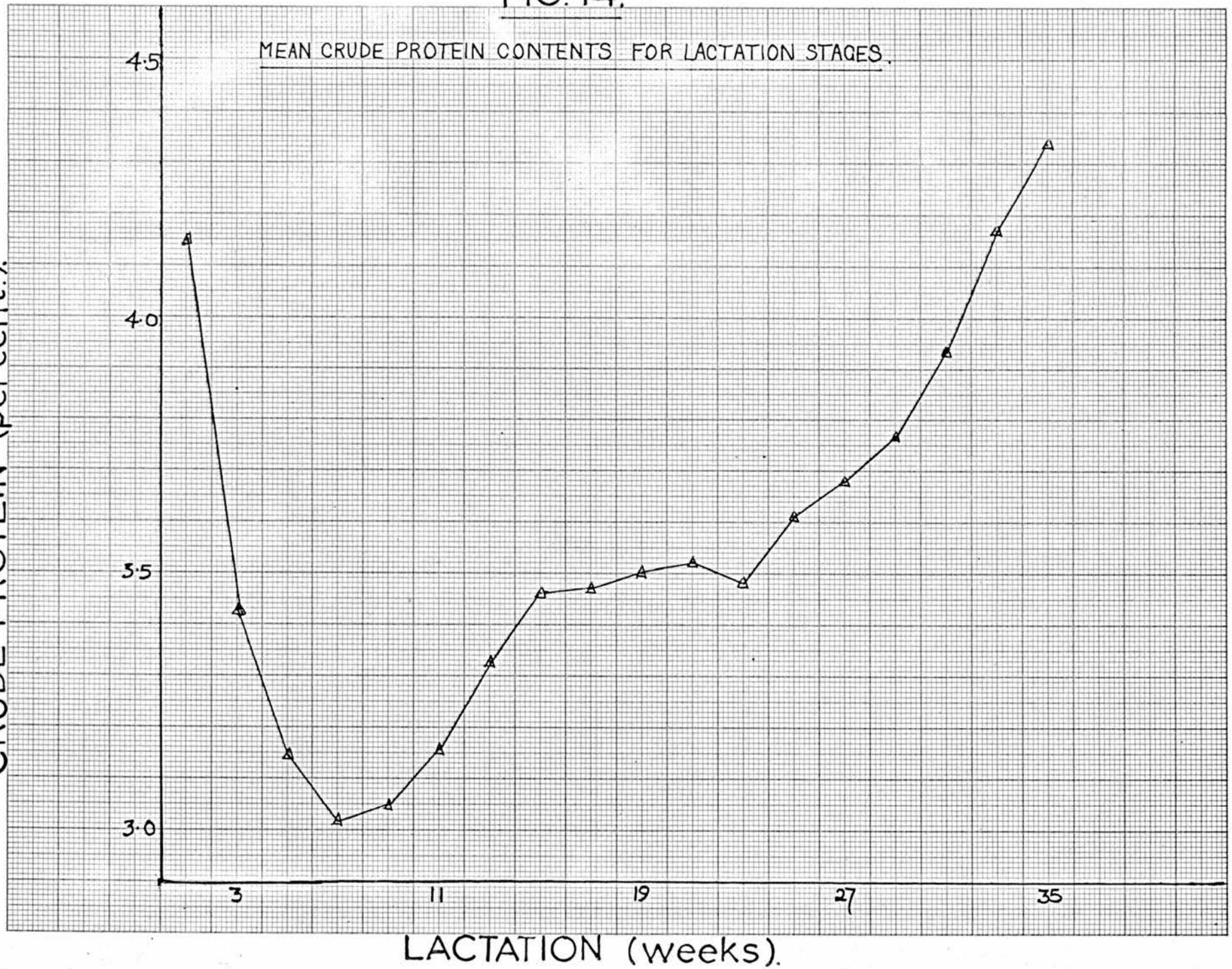

MEAN LACTOSE YIELDS FOR LACTATION STAGES.
LACTOSE YIELD (lb.).
2·5
2·0
1·5
1·0
0·5
3
11
19
27
35
LACTATION (weeks).

FIG. 14.


MEAN CRUDE PROTEIN CONTENTS FOR LACTATION STAGES.
4·5
4·0
3·5
3·0
CRUDE PROTEIN (percent.).
3
11
19
27
35
LACTATION (weeks).

lactose content is low in early lactation, rises to a maximum at about the period of peak yield and then falls to the end of the lactation.

A study of the figures in the relevant appendix tables shows very considerable differences in the exact shape of the lactation curve for individual cows and an analysis of variance showed that both cow and stage of lactation had a significant effect. Mean lactose values for the five cows are plotted in Fig. 12. The correlation with yield is not close. Maximal lactose content is not attained until the seventh week post-partum and then remains almost constant to the seventy seventh day before starting a decline which accelerates to the end of lactation. Lactose production shows a lactation curve very similar to that for yield (Fig. 13).

Crude Protein Content The figures in the appendix tables show wide variation in level of protein, and the shape of the lactation curve for protein content for individual cows. Analysis of variance provides significant evidence for differences between cows and between lactation stages. Mean values at different stages throughout the lactation are shown in Fig. 14. The value is high in early lactation but drops sharply to the thirty-fifth day and reaches a minimum at day forty nine. There is then a sharp rise to the hundred and fifth day, followed by a comparatively short period of little change before a rise from the hundred and eighty-first day. The curve is different in shape from those for the other constituents. Crude protein production figures show significant cow and stage of lactation effects. Mean values over the lactation period are given

FIG. 15.

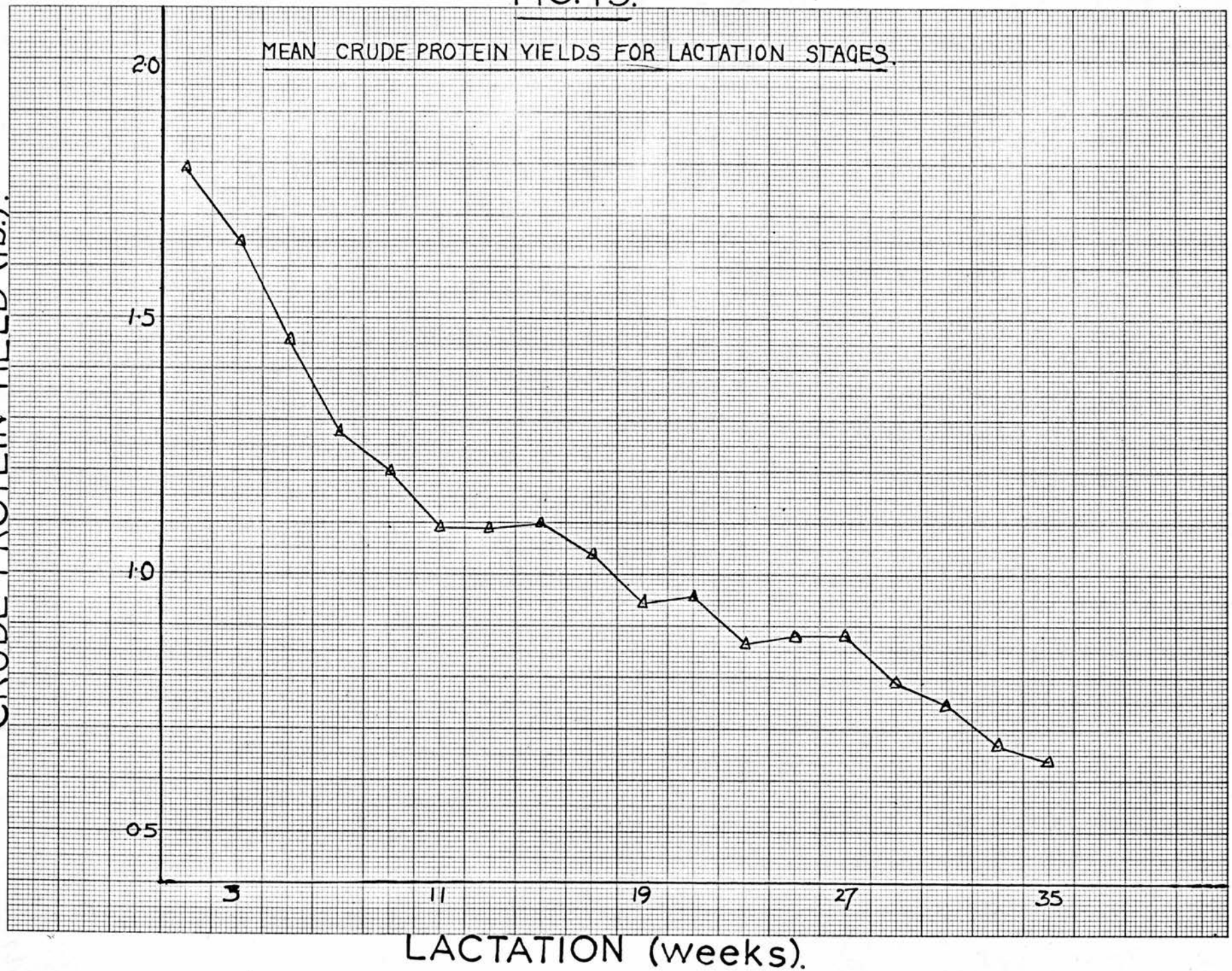

MEAN CRUDE PROTEIN YIELDS FOR LACTATION STAGES.
CRUDE PROTEIN YIELD (lb.).
2·0
1·5
1·0
0·5
3
11
19
27
35
LACTATION (weeks).

FIG.16.

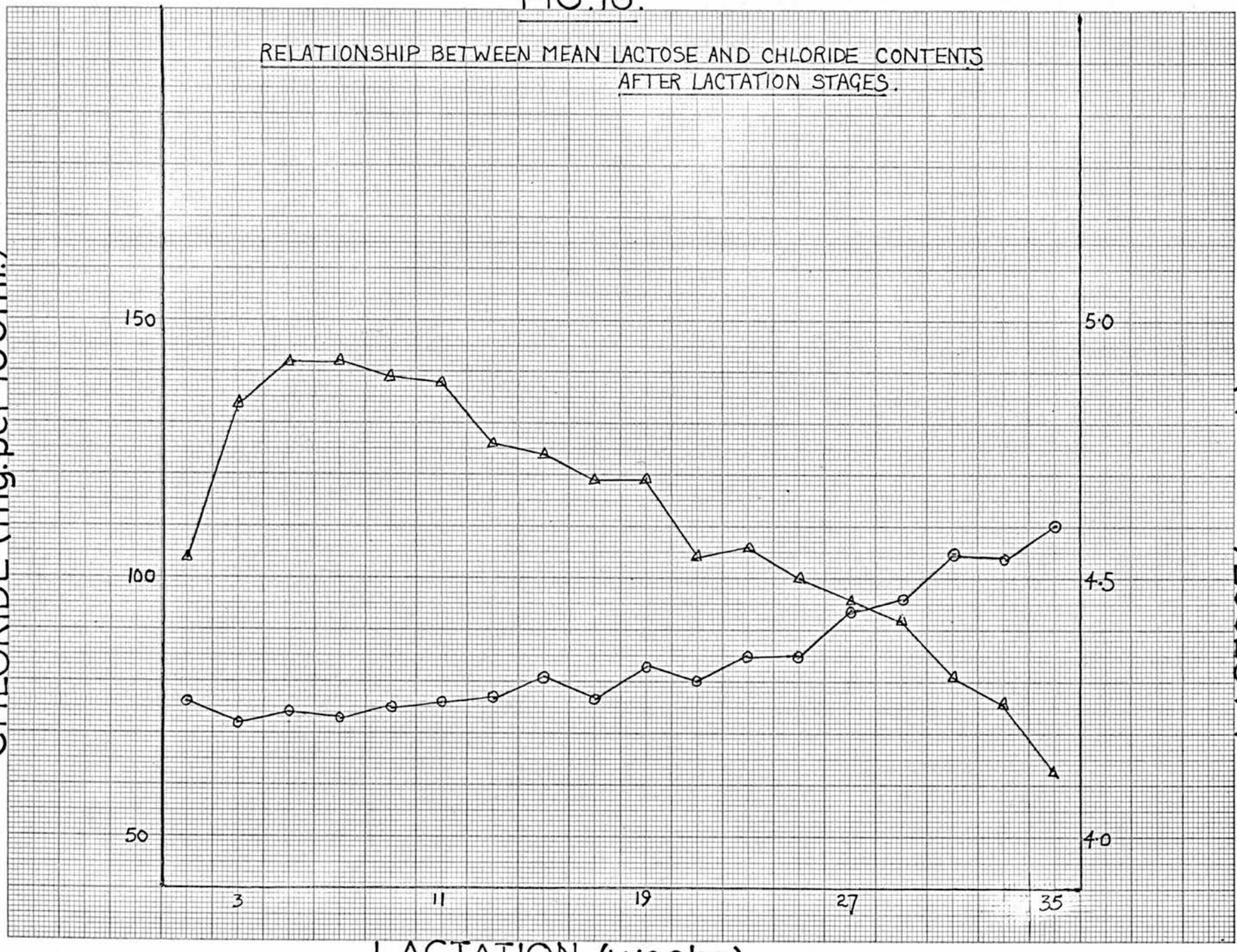
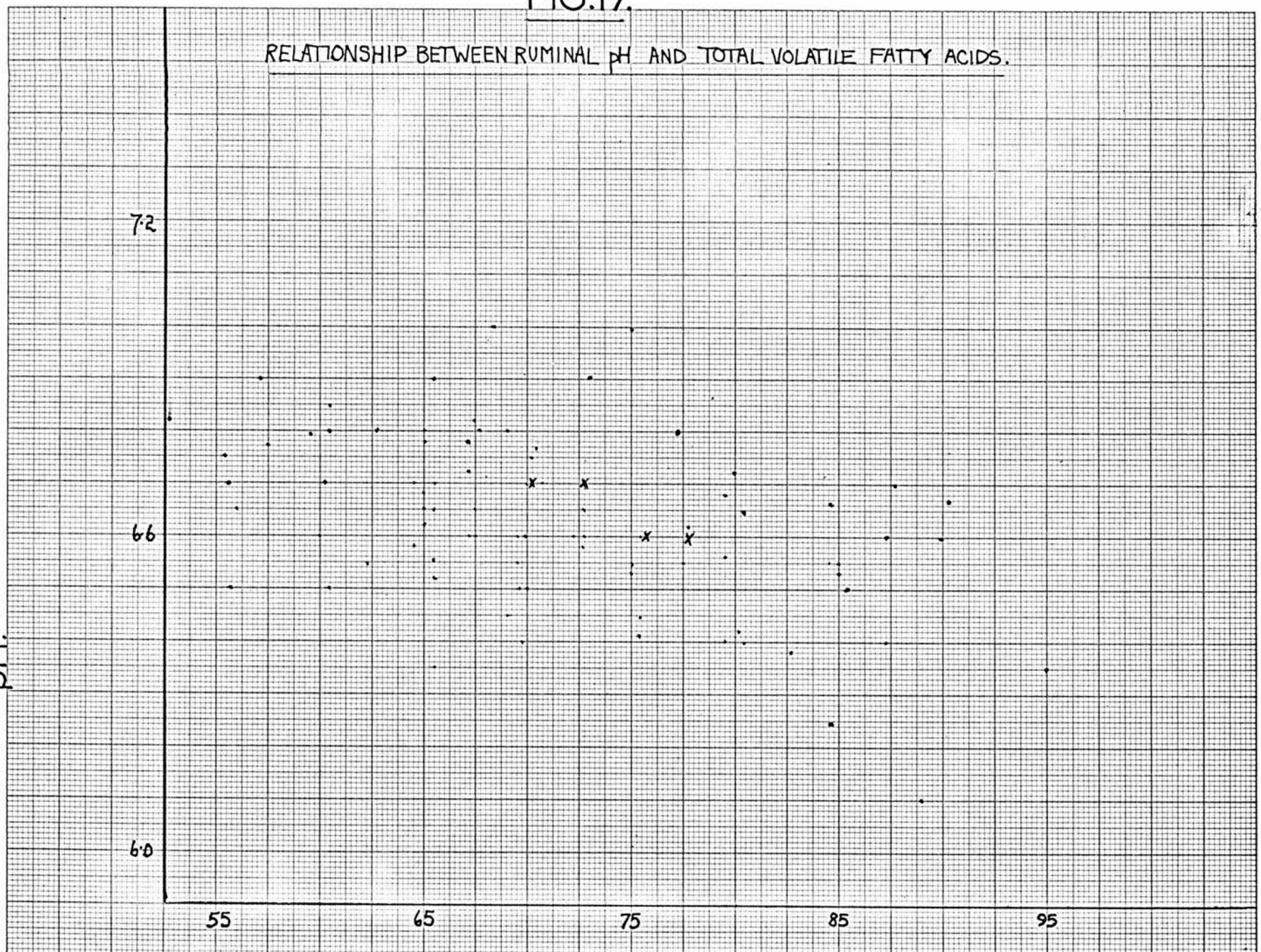

RELATIONSHIP BETWEEN MEAN LACTOSE AND CHLORIDE CONTENTS AFTER LACTATION STAGES.
CHLORIDE (mg.per100ml.)
150
100
50
5·0
4·5
4·0
LACTOSE (percent.).
3
11
19
27
35
LACTATION (weeks).

FIG.17.


RELATIONSHIP BETWEEN RUMINAL pH AND TOTAL VOLATILE FATTY ACIDS.
7·2
6·6
6·0
pH.
55
65
75
85
95
T.V.F.A. (m mole per litre).

in Fig. 15 which shows a fall in production throughout with a mid-lactation period showing less change.

Chloride Content Chloride content was determined as a check on the state of the udder. An analysis of variance showed significant differences between cows and between stages of lactation. The mean chloride figures for different stages of lactation are shown in Fig. 16, along with those for mean lactose content. Except at the end of the lactation there appears to be no correlation between the two. The chloride content is remarkably constant throughout the major part of the lactation. Only on seven occasions did the chloride content exceed 115 mg./100 ml. and these values were obtained in late lactation, where lactose and chloride values showed a close negative correlation.

Rumen Contents

The analytical results for the rumen liquor samples are given in Appendix Tables 3, 7, 11, 15, 19 and 23.

pH The range of pH values recorded was 6.10 to 6.95 with a general mean of 6.63. Analysis of variance showed no significant evidence of a difference between cows or between stages of lactation.

Total Volatile Fatty Acids. The range of values was 52.5 to 95.0 m.mole/litre with a general mean of 71.9. Analysis of variance showed no significant evidence of a difference between cows or between stages of lactation. The relationship between the concentration of total volatile fatty acids and pH is shown in Fig. 17. There is no correlation within the range covered.

Individual Volatile Fatty Acids The acids found to be present were acetic, propionic, normal butyric, iso-butyric, iso-valeric and normal-valeric. No caproic acid was detected in any of the samples. Analyses of variance gave significant evidence for

a difference between cows in the content of propionic acid (P = 0.05) iso-butyric acid (P = 0.01) and iso-valeric acid (P = 0.001) but in no case was there evidence for a difference in the content of any of the acids at different stages of lactation.

The proportions of the different fatty acids stated as molar percentages of the total volatile fatty acid content are shown in Appendix Tables 4, 8, 12, 16 and 20. Analyses of variance gave significant evidence for differences between cows for the proportions of propionic acid (P = 0.01), iso-butyric acid (P = 0.05) and iso-valeric acid (P = 0.001). There was no evidence for any differences between stages of lactation. Mean values for the amounts and proportions of the individual acids are given in Table 5.

Table 5

Mean Concentration and Molar Percentages of Individual Volatile Fatty Acids in Rumen Liquor

	Molar percentage	M.mole./litre
Acetic acid	65.53	44.51
Propionic acid	15.27	10.76
Butyric acid	1.69	10.96
Iso-butyric acid	15.75	1.19
Iso-valeric acid	2.24	1.60
n-valeric acid	1.49	1.07

Acetone + Acetoacetic Acid This fraction was present in all but eleven of the samples of rumen liquor examined. The range of values extended from 0 - 11.07 mg./100 ml. of acetone with a mean value of 2.61. The variation was considerable and analysis of variance gave no significant evidence of differences between cows or between stages of lactation. Mean figures for acetone plus

FIG.18.

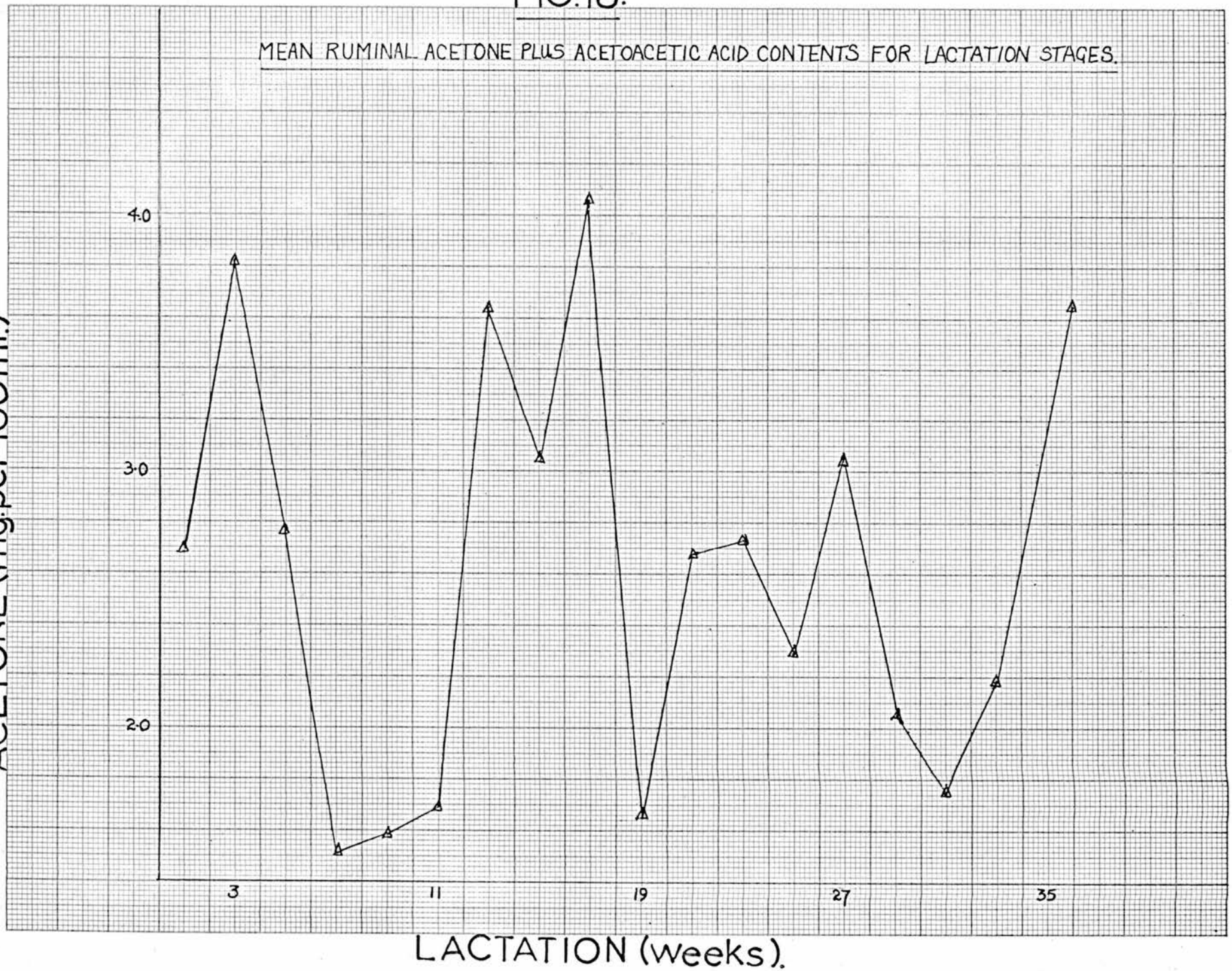

MEAN RUMINAL ACETONE PLUS ACETOACETIC ACID CONTENTS FOR LACTATION STAGES.
ACETONE (mg.per100ml.)
4·0
3·0
2·0
3
11
19
27
35
LACTATION (weeks).

FIG.19.

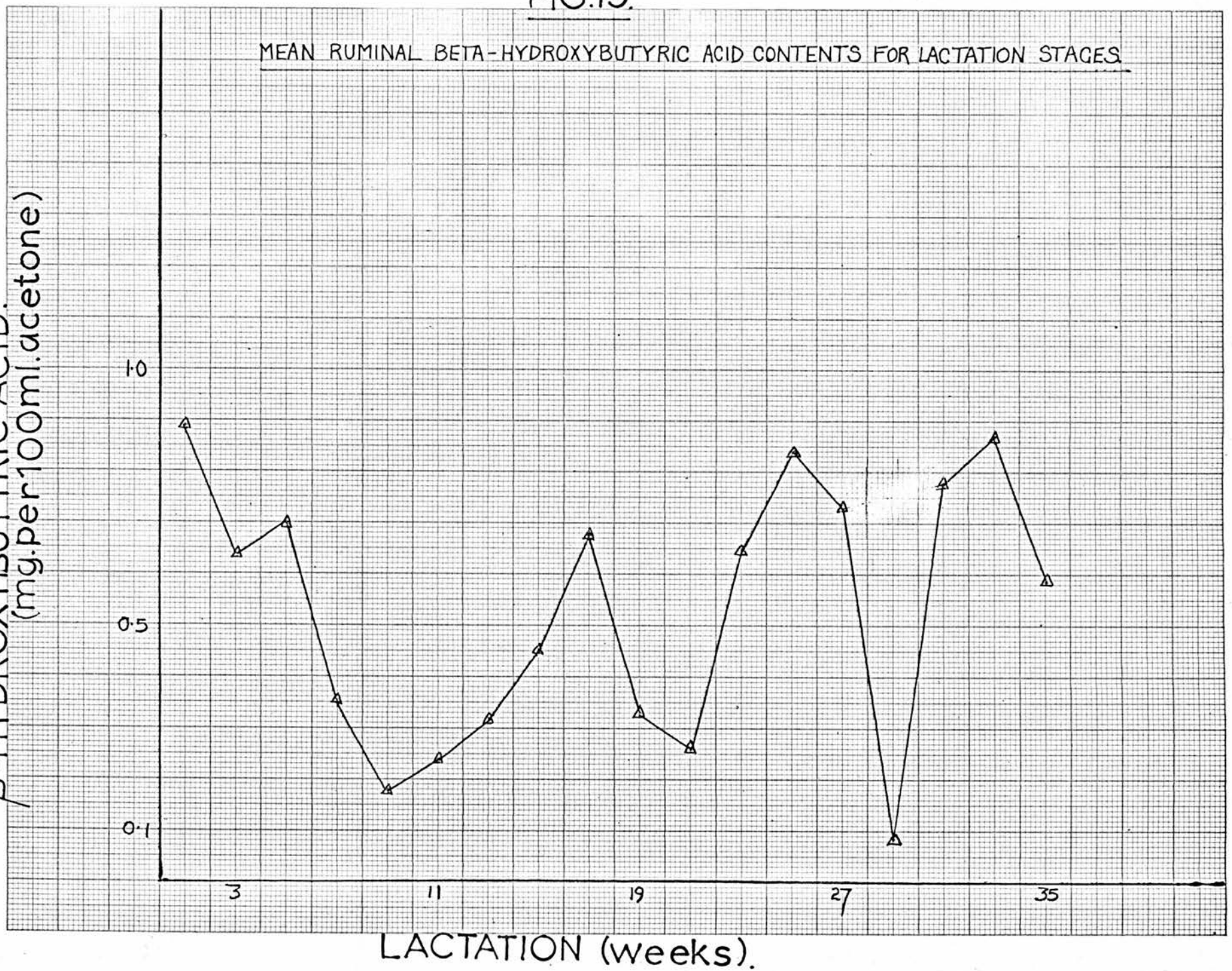

MEAN RUMINAL BETA-HYDROXYBUTYRIC ACID CONTENTS FOR LACTATION STAGES.
B-HYDROXYBUTYRIC ACID. (mg.per100ml.acetone)
1·0
0·5
0·1
3
11
19
27
35
LACTATION (weeks).

FIG. 20.

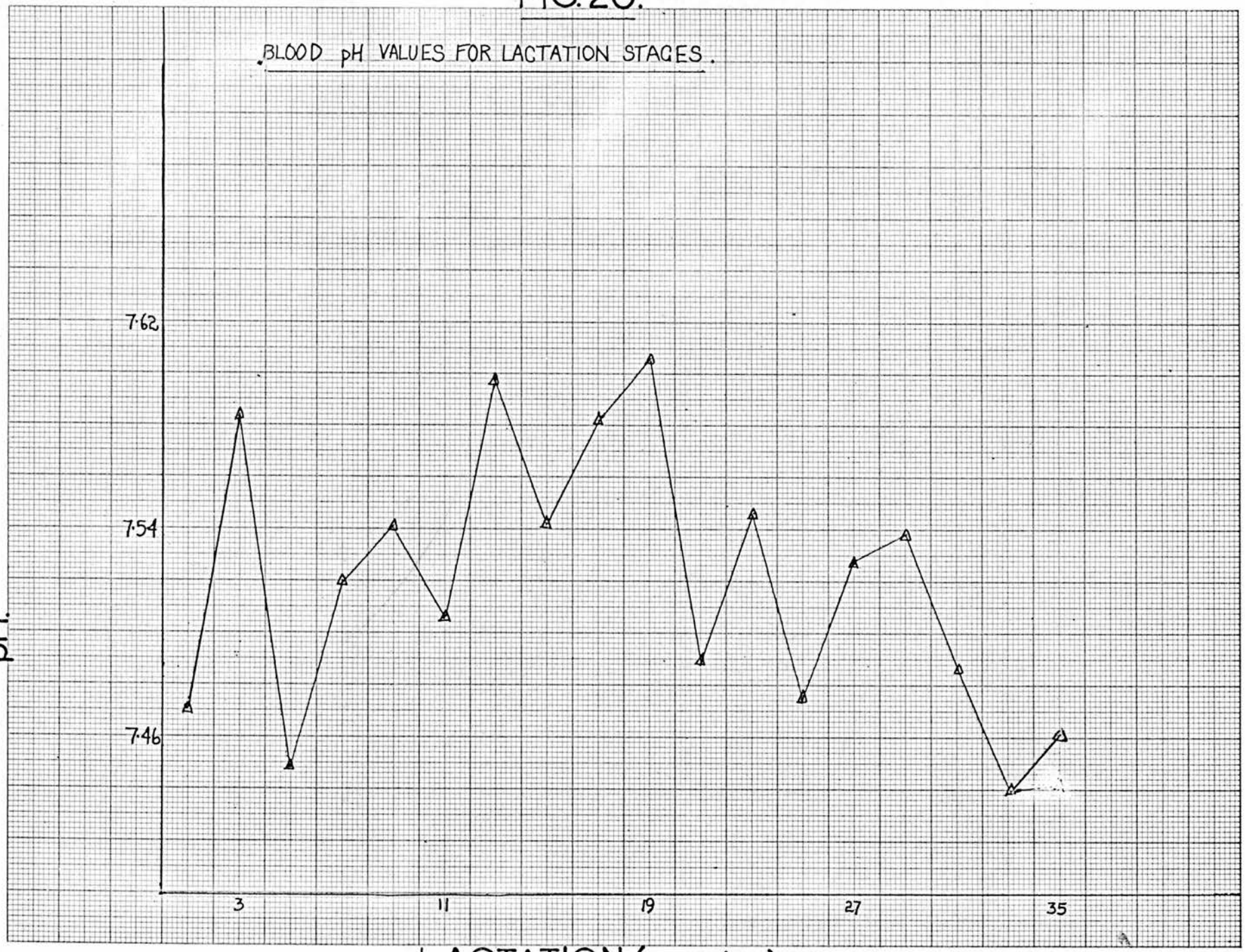
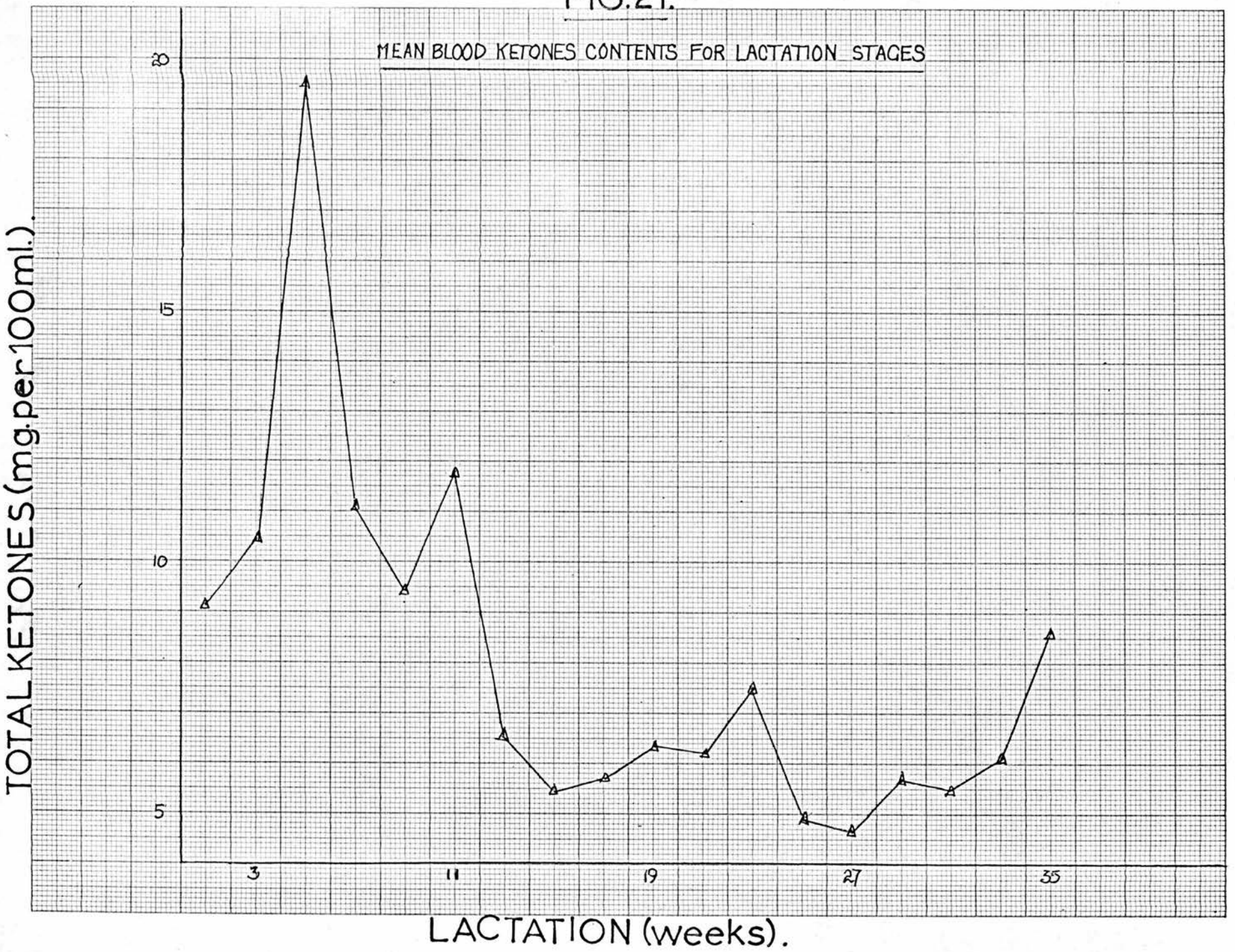

BLOOD pH VALUES FOR LACTATION STAGES.
7·62
7·54
7·46
pH.
3
11
19
27
35
LACTATION (weeks).

FIG.21.


MEAN BLOOD KETONES CONTENTS FOR LACTATION STAGES
TOTAL KETONES (mg. per 100ml.).
20
15
10
5
3
11
19
27
35
LACTATION (weeks).

acetoacetic acid at different stages of lactation are given in Fig. 18.

Beta-hydroxybutyric acid Values ranged from 0 - 1.60 mg./100 ml., as acetone, with a mean value of 0.53. There was significant evidence of differences between stages of lactation but not between cows. Mean values for five cows at different lactation stages are plotted in Fig. 19. There is no discernible lactation trend.

Blood

The analytical results for the blood samples are given in Appendix Tables 2, 6, 10, 14, 18 and 22.

pH The range of pH values recorded was 7.38 to 7.74 with a mean of 7.52. An analysis of variance gave significant evidence for differences between cows and between stages of lactation. The mean pH figures for the five cows over the lactation period are shown in Fig. 20. There is no lactation trend.

Total Ketones The range of values is 1.55 to 33.79 mg./100 ml., as acetone, with a mean value of 8.0 mg./100 ml. Analysis of variance gave significant evidence for differences between cows ($P = 0.01$) and between lactation stages ($P = 0.001$). Mean values for the five cows at different stages of lactation have been used to construct Fig. 21. Total ketone content rises to a maximum at week five and then falls to week thirteen, remaining almost constant thereafter.

Acetone plus Acetoacetic Acid The range of values is from 0 to 11.25 mg. acetone per 100 ml. with a mean of

FIG. 22.

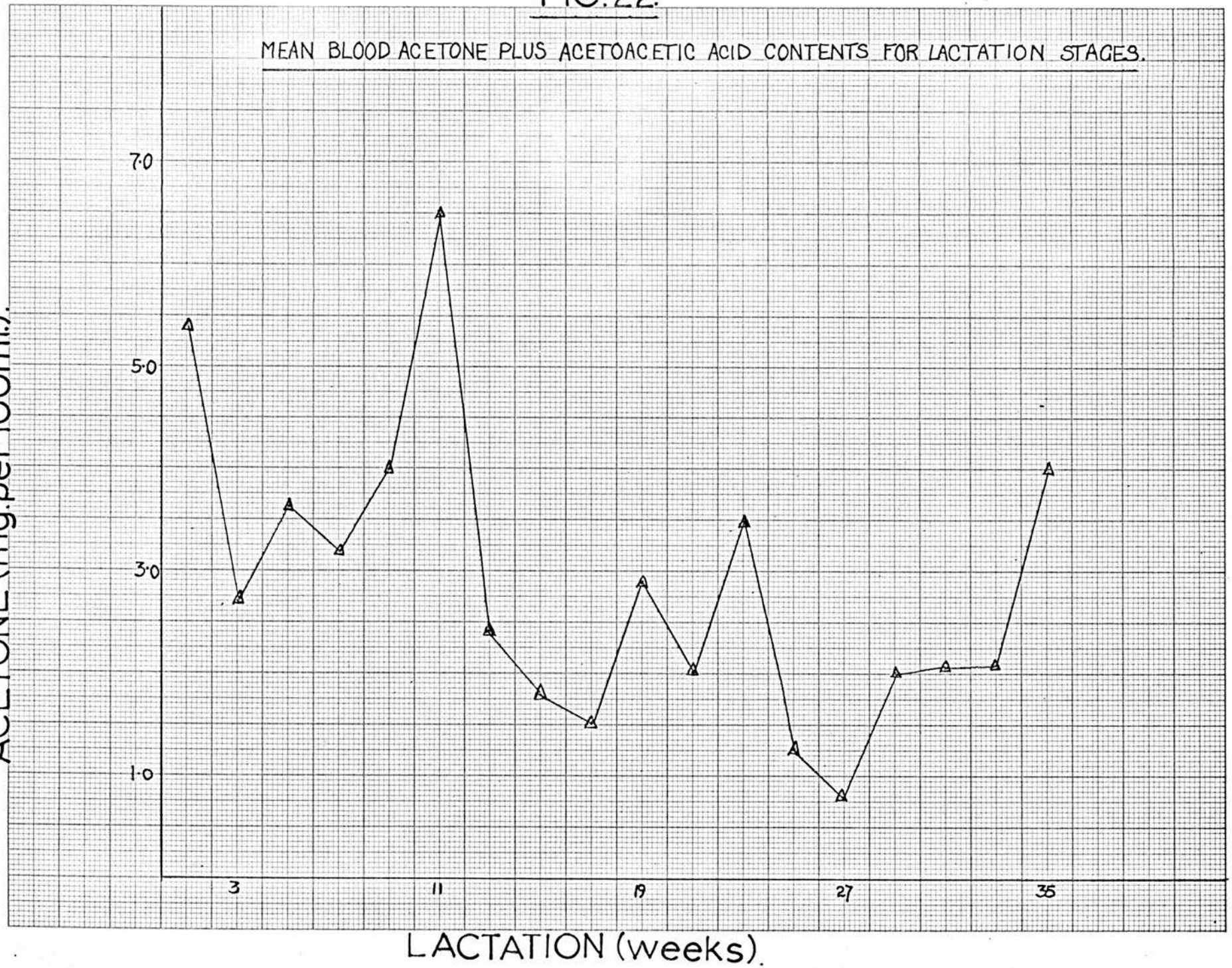
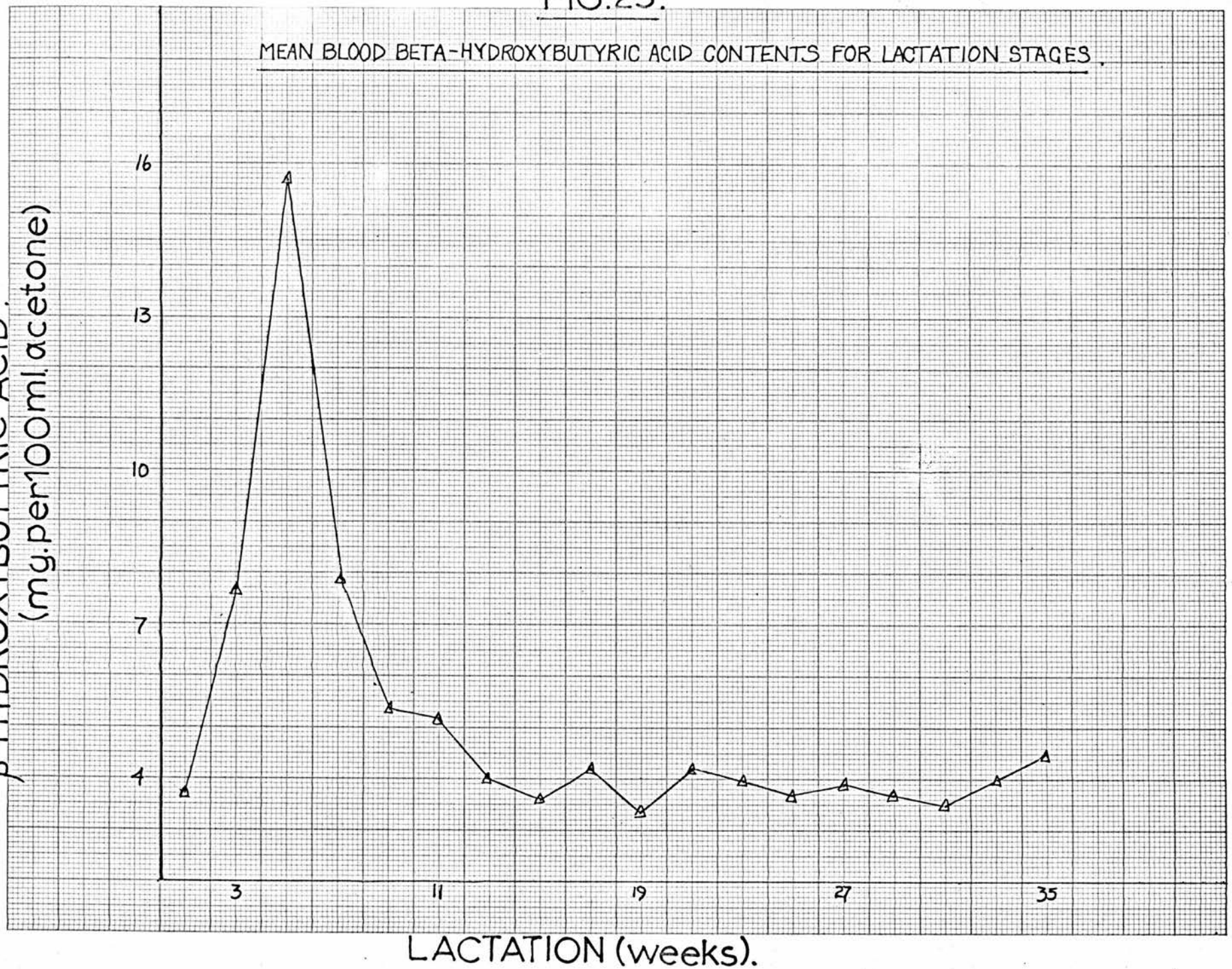

MEAN BLOOD ACETONE PLUS ACETOACETIC ACID CONTENTS FOR LACTATION STAGES.
7·0
5·0
3·0
1·0
ACETONE (mg. per 100 ml.).
3
11
19
27
35
LACTATION (weeks).

FIG.23.


MEAN BLOOD BETA-HYDROXYBUTYRIC ACID CONTENTS FOR LACTATION STAGES.
β-HYDROXYBUTYRIC ACID. (mg.per100ml.acetone)
16
13
10
7
4
3
11
19
27
35
LACTATION (weeks).

2.9 mg./100 ml. Mean values for the five cows for the different stages of lactation are shown in Fig. 22. There is a fall to the third week post-partum and the level then remains relatively constant before rising sharply at week eleven. By the thirteenth week the level has fallen again and there is little change thereafter until the final stage of lactation when it rises. An analysis of variance showed the value at the eleventh week post-partum to be significantly higher ($P = 0.05$) than at any other except weeks one, nine and thirty five. Of the five cows, three showed a rise at this stage but two showed a sharp drop and it is doubtful if too much weight should be placed on this as a lactation effect.

Beta-hydroxybutyric acid The data show a range of values from 0.8 to 27.4 mg./100 ml., as acetone, with a mean of 5.1 mg./100 ml. Analysis of variance gave significant evidence of defferences between cows ($P = 0.01$) and between stages of lactation ($P = 0.001$). Mean values for the beta-hydroxybutyric acid content of the blood of the five cows over the lactation period are shown in Fig. 23. The curve rises sharply to a maximum at five weeks post-partum and then declines equally sharply and from the ninth week post-partum shows little or no change. This lactation pattern is common to all five cows although the times at which the maxima occur vary, in two cases being at the seventh and in the other three at the fifth week post-partum. The mean levels at weeks three, five and seven post-partum are significantly higher than the rest of the lactation.

Total Volatile Fatty Acids The total volatile fatty acid contents cover a range of 0.28 to 1.62 m.mole/litre with a

FIG. 24.

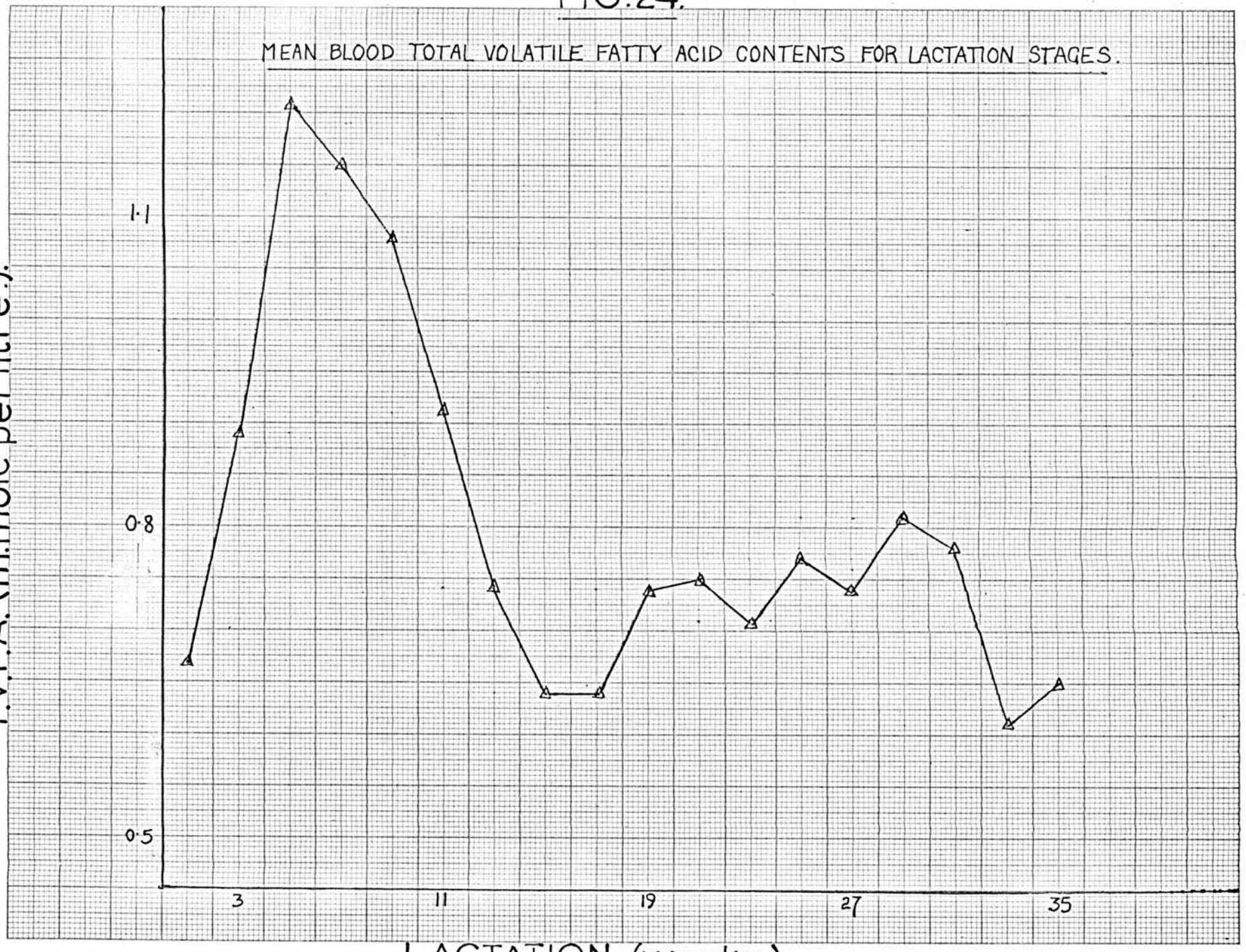
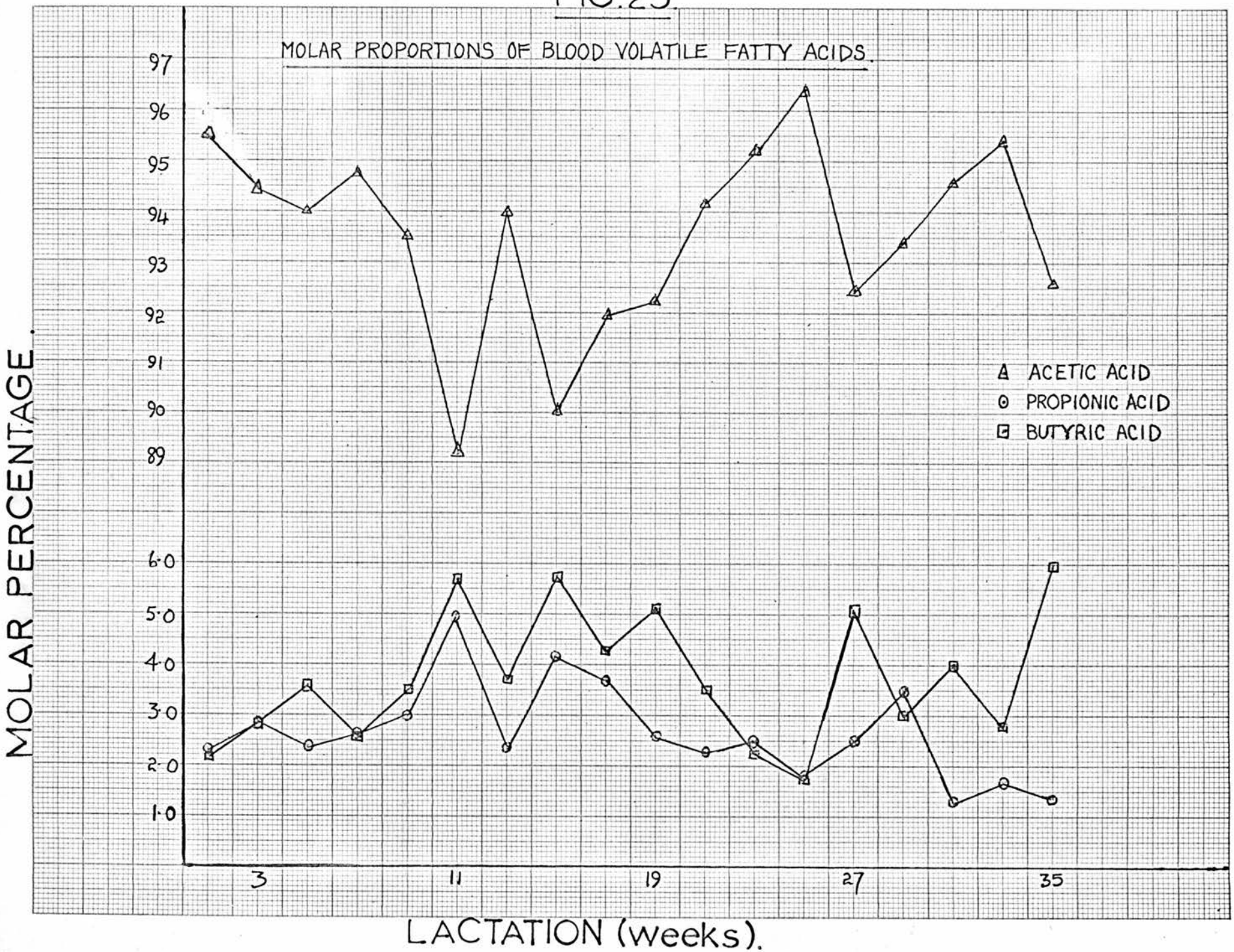

MEAN BLOOD TOTAL VOLATILE FATTY ACID CONTENTS FOR LACTATION STAGES.
T.V.F.A. (m.mole per litre.).
1·1
0·8
0·5
3
11
19
27
35
LACTATION (weeks).

FIG. 25.


MOLAR PROPORTIONS OF BLOOD VOLATILE FATTY ACIDS.
97
96
95
94
93
92
91
90
89
6·0
5·0
4·0
3·0
2·0
1·0
ACETIC ACID
PROPIONIC ACID
BUTYRIC ACID
3
11
19
27
35
MOLAR PERCENTAGE.
LACTATION (weeks).

mean value of 0.80 m.mole/litre. Analysis of variance gave significant evidence (P = 0.001) of differences between cows and between stages of lactation. Figures for all five cows agree in showing a rise in early lactation followed by a fall to a period of relatively constant composition which lasts for the rest of the lactation. The lactation curve based on mean figures for the five cows is shown in Fig. 24 with a maximum at week three which is maintained until week nine after which there is a sharp drop to the period of constant composition.

Individual Volatile Fatty Acids The figures in the appendix tables are given as molar percentages as this was thought to be more meaningful in view of the low levels of propionic and butyric acids present in the blood. Analysis of variance gave no evidence of differences between cows in the proportions of the individual acids present but there was significant evidence (P = 0.001) for differences between stages of lactation for all three acids. Mean values for the three acids at different stages of lactation are shown in Fig. 25. Changes are sporadic and there is no discernible lactation trend. Mean values for the molar percentages are 93.6, 2.7 and 3.8 for acetic, propionic and butyric acids respectively. This means that a value for blood total volatile fatty acids is, to all intents and purposes, a value for acetic acid content.

DISCUSSION

Cannon et al[115] showed a fall in yield from the first month of lactation, the decline accelerating throughout the lactation. Most workers have shown a rise to peak yield at an early stage of lactation

but the time at which this took place varied from thirty to eighty days post-partum. Waite et al[116] working with the Ayrshire breed under Scottish conditions, provide the work most comparable to the present investigation. They showed maximum yield at about the forty-fifth day post-partum. Rook and Campling[132] worked with six Friesian cows kept under carefully controlled conditions of environment, management, feeding, and udder infection. Changes during lactation therefore represented those due to physiological factors as with the present investigation. They showed a rise in yield to a maximum at twenty one days which was maintained until thirty five days and then fell slowly to about thirty five weeks when the rate of decline increased. The information presented here agrees with the findings of Rook and Campling[132] in showing the early maximum yield, but the mid-lactation period shows more change and in this way is more akin to the data of Waite et al[116] and Cannon et al.[115] The non-constant rate of decline of yield indicates that the widely held view that yield declines by about 2.5 per cent per month is untenable.

The lactation curve for fat content in the present investigation showed the same general shape as that agreed by most previous workers. The fall to a minimum occurs earlier than in the work of Waite et al[116] by about ten days but the rise thereafter is much quicker and at seventy seven days there begins a period of one hundred and thirty days when fat content remains practically constant before the late lactation rise. Due to the slow rise from the minimum, the period of constant fat content in Waite's data is only about thirty days. Rook and Campling[132] agree with the present work in that the minimum value occurs at thirty five days and there is a long mid-lactation

period of constant fat content. The rise from the minimum period is not as well defined as in the present data.

The lactation curve for solids-not-fat content differs from that of Waite et al[116] in having a minimum value at about sixty three days instead of forty five days, but recovery is quicker and there is a comparable mid-lactation period of about a hundred days when composition is constant, before the late lactation rise. The data of Rook and Campling[132] show a minimum value at thirty five days but no recovery, the level of solids-not-fat remaining practically constant until about thirty five weeks when the late lactation rise takes place.

The total solids content being the sum of the solids-not-fat and fat contents shows the same lactation pattern with the rises and falls accentuated.

The lactation curve for crude protein content shows a minimum at thirty five days, some 10 days earlier than that in Waite's[116] data, but whereas in the latter there follows a rise to the end of lactation the present data show a sharp rise followed by a period of little change until the late lactation rise begins. Rook and Campling[132] agree with the early minimum and rise to one hundred and five days but there is a much longer period of constant protein content.

The lactose curve Fig. 12 agrees with that of both Rook and Campling[132] and Waite et al[116] in showing an increase from the early lactation level. The maximum at thirty five days agrees with the former but is ten days later than that given by Waite et al.[116] The

latter showed a decline from the maximum until a sharper late lactation drop whereas Fig. 12 shows a plateau lasting for about sixty five days and there is then a drop to a level which is maintained for a further 50 days before the late lactation drop. The data of Rook and Campling[132] showed little or no change from the maximum lactose content until the late lactation drop.

There is considerable agreement between this data and that of Waite et al[116] for the same breed of cow, the main differences being in the earlier minima and maxima and the mid-lactation constancy of composition which is hardly noticeable in Waite's data. In this the results agree with those of Rook and Campling[132] and this probably reflects the similar derivation of the results. The differences between the two sets of results are probably due partly to a breed effect and partly to the different time at which the animals were bred. In both sets of data the late lactation rises or falls took place about twenty weeks after conception but were separated by a period of about fifty to sixty days. The more marked recovery from minimum values shown in these data compared with those of Rook and Campling is difficult to explain.

The chloride content of the milks analysed show a surprising lack of correlation with lactose content in the early parts of lactation but later there appears to be a close inverse relationship. Rowland and Zein-El-Dine[133] in an examination of indirect methods of detecting udder infections considered chloride content to lie next to Casein No. in efficiency. They suggested that 115 mg./100 ml. of chloride should be the standard for detecting infections. Only in the later stages of lactation, were chloride figures over 115 mg./100 ml. obtained in the

present investigation. High figures would be expected at this stage to balance the late lactation fall in lactose content. It was concluded that the influence of udder infections on the data was negligible.

Eusebio et al[71] found a range of total volatile fatty acids from 103.8 to 140.3 m.mole/litre for diets of different kinds. Surprisingly the two diets giving the values quoted were almost exactly similar at 13lb. hay + 5.7lb. maize meal. A diet of flaked maize alone gave a total volatile fatty acid content of 125 m.mole/litre. Schultz[72] examined rumen liquors from normal and ketotic cows on a winter diet and found total volatile fatty acid concentrations of 74.6 compared with 102.8 m.mole/litre. Brown and Shaw[74] fed diets containing about 17 lb. hay + concentrates to normal and ketotic animals and found total volatile fatty acid concentrations of 88.8 compared with 119.8 m.mole/litre and gave 59.4 m.mole/litre for normal fasted animals. Balch & Rowland[134] gave values of 112 to 93 m.mole/litre for diets ranging from all-hay to high-concentrate. Henders and Ward[135] quote a figure of 54.3 m.mole/litre of total volatile fatty acids for a diet of hay and concentrates. Bath and Rook[122.136] gave values of 90 to 115 m.mole./litre for hay/concentrate diets in one series of experiments and 83.4 - 100.2 in another. Storry and Rook[137] fed a diet of 8 Kg. hay + 10 kg. concentrate and changed to one containing only 1 kg. hay and found total volatile fatty acid concentration changed from 140 to 120 m.mole/litre. The levels of total volatile fatty acids found in the present experiment appear to be low compared with the majority of reports in the literature. Only

ketotic or fasted cows approach the lower order of the range. It is difficult to account for this as on no occasion did the cows exhibit clinical signs of ketosis and always consumed a full ration of food. Furthermore rumen liquor samples were taken at 3-hours after the concentrate feed when acid production in the rumen should have been maximal.

The relationship between pH and total volatile fatty acid concentration has been the subject of considerable controversy. Coop[138] in studies with sheep showed a close correlation in animals fed after a fast but was unable to show this in fed sheep over several days. Cason et al[139] considered rumen pH to be related to the ash content of the digesta. Phillipson[140] considered rumen organic acids to be related to pH but that lactic acid had a very marked effect. Both Chance et al[141] and Briggs et al[142] considered pH to be related to volatile fatty acid concentration. There was no supporting evidence for this in the present work. This may be accounted for by a combination of dilution and excessive salivation, which can sometimes be provoked by the method of sampling used, and also by variation in the lactic acid content of the rumen liquor.

A wide range of volatile fatty acids has been recorded in rumen contents on different diets. Gray et al[76] fed sheep on wheaten hay and reported acetic, propionic, butyric, iso-butyric, normal-valeric, caproic and heptanoic acids to be present. Acetic, propionic and normal butyric acids made up about 95 per cent of the total volatile fatty acid content. Annison[78] fed sheep on diets containing high levels of groundnut cake, maize and casein and reported the presence

of iso-valeric and 2-methyl butyric acids but no caproic and no heptanoic acids. Annison suggested that the branched chain and valeric acids were produced by deamination of amino acids. El Schazly[77] found acetic, propionic, normal butyric, iso-butyric, iso-valeric, normal-valeric and caproic acids in the rumen contents of sheep fed grass and silage. A casein supplement increased the concentration of the minor acids. In the present investigation no caproic acid was found although this has been found by the author in rumen contents in numerous other cases and is indeed reported later in this work. The mode of formation of caproic acid is probably by bacterial action on lactic acid. The organism involved is Peptostreptococcus elsdenii[143] and conditions may not have been suitable for its activity in the present investigation. 2-methyl-butyric acid was not found and this is difficult to account for since its precursor[78] iso-leucine is present in adequate amounts in the protein sources provided for the animals.

Acetic acid is the major acid present, the proportion (stated as a molar percentage) being similar to those quoted by Tyznik and Allen[69] 65.0 per cent, Ensor et al[70] 61.9 per cent, Eusebio[71] 60.3 per cent, and Brown and Shaw[74] 65.3 per cent. Bath and Rook[122] gave figures varying from 57.8 to 67.8 per cent for cows fed on hay plus concentrate diets. The range of values in the present data agrees very well with their results. The diet fed to the cows is traditional and very common for dairy cows. It is interesting that in practically every case the molar percentage of acetic acid is within the range of 50 to 65 per cent, quoted by Blaxter[144] as allowing maximal energy utilisation for milk production.

The molar percentage of propionic acid in the rumen contents

is lower than would be expected from the literature. Thus the present mean is 15.27 per cent compared with 20 per cent,[69] 20.2 per cent,[70] 21 per cent,[71] 20.6 per cent,[74] 19.5 per cent.[122] The figures of 16.1 and 16.9 per cent given by Bath and Rook[136] for cows fed ryegrass hay and concentrates are more in keeping with those reported here. It is interesting that they gave 17.1 and 24.4 per cent for comparable Timothy/Meadow Fescue diets. Generally speaking the proportion of normal butyric acid in the volatile fatty acids of the rumen contents is less than that for propionic although there is considerable variation from author to author. Tyznik and Allen[69] quote 15 per cent for normal diets and when acetic acid fell on low roughage diets showed an increase in propionic acid but the butyric acid remained constant. For diets comparable with ours Eusebio[71] gave 13.7 per cent, Ensor[70] 9.0 per cent, Brown[74] 14.1 per cent and Bath and Rook[122] 11.6 per cent. Later,[136] for ryegrass hay they gave 14.8 and 13.5 per cent compared with 8.6 and 9.0 for a Timothy hay and concentrate diet. Any decreases in acetic acid in the work of the above workers was accompanied by a rise in the propionic acid content while butyric acid remained constant. In the present work this was not so. Decreases in the molar percentage of acetic acid could equally well be balanced by a rise in the molar percentages of both propionic and normal butyric acids or by a rise in one or the other. Thus butyric acid showed a higher molar percentage than propionic acid on 54 out of 90 recorded tests. The relatively equal quantitative importance of butyric and propionic acids shown in the present work is at variance with the experience of other workers but

some support is lent by the work of Bath and Rook[136] which indicates that the nature of the hay fed may have an effect. The hay here was from an almost pure ryegrass stand. On certain types of diet butyric acid may be of greater importance as an energy source than is generally thought. Armstrong[145] states that on a molar basis acetic acid only supplies 40 per cent of the energy that butyric acid does and draws attention to the fact that much of it enters the peripheral circulation as beta-hydroxybutyric acid which is available to the mammary gland for fat synthesis.

Thin and Robertson[94] examined the rumen liquors of normal and ketotic cows and found a range of total ketone bodies from 0 - 10.25 mg. acetone per 100 ml. with a mean of 3.26 mg/100ml. for those of normal animals. For ketotic animals they quote a range of 2.11 to 55.4 mg./100 ml. with a mean of 20.06 mg./100ml. These figures agree very well with those of 0.02 to 11.12 mg/100 ml. and 3.16 mg/100 ml. recorded in the present investigation. Thin and Robertson[94] stated that neither acetone nor acetoacetic acid were present in the rumen liquors of normal cows and that the increase in ketone bodies in ketotic cows was due to increasing amounts of this fraction relative to beta-hydroxybutyric acid, which was the only ketotic constituent in normal liquors. The presence of an acetone + acetoacetic acid fraction in all but ten of the rumen liquors examined here is not taken as evidence that the animals were suffering from ketosis. In fact there were no clinical signs at any time during the whole experimental period. The methods of analysis used in the two investigations were of course different.

None of the constituents of the rumen liquor showed any significant variation over the lactation period except for beta-hydroxybutyric acid and no lactation trend was discernible even here. Only the amounts and proportions of iso-butyric, iso-valeric and propionic acids showed between cow differences. This absence of significant differences must not be taken to argue a constancy of composition. Rather, it is the consequence of large standard errors for the data. Considerable differences do exist at different stages and with different cows.

The concentrate intake during the experimental period varied from about eight to twenty pounds per day. Although this was a gradual change taking place over a long period no corresponding changes took place in the rumen contents. It would appear that with a basic ration of hay at the level fed here, then quite large variations in concentrate intake do not affect the nature of the rumen fermentation. Bath and Rook[136] reached much the same conclusion.

The lack of correlation between changes in the rumen constituents and changes in milk yield and composition is indicative of a lack of influence by rumen changes on the latter. It is reasonable to conclude that the lactation changes recorded in milk yield and composition were physiological in origin.

Krapf[146] gave a range of 7.35 to 7.50 for the pH of cow's blood. The range shown in the present data is wider and shows pH at a higher level. The present results do confirm, however, the remarkable constancy of the pH of blood, which is maintained by its high buffer capacity.

The levels of ketones in blood are very much higher than

those in rumen contents, by a factor of 2½. This is in agreement with the findings of Thin and Robertson[94] who gave a lower mean value of 6.10 mg. acetone per 100 ml. and a narrower range of 2.87 to 10.88 mg/100 ml. Bach and Hibbit[95] quoted almost exactly similar figures to those of Thin and Robertson and noted that total ketone levels were high in early lactation. Shaw[96] dealing with the immediate post-partum period showed a large increase in blood ketones within one to three days after calving and levels up to 70 mg/100 ml. were quoted. Schmidt and Schultz[101] showed high blood ketones, of the order of 12 mg/100 ml., at three weeks post-partum, the high levels being maintained for a very short period only. Knodt et al[120] took blood samples from eleven cows at thirty day intervals over a twelve month period. They showed a slight rise in total ketones to the 90th day post-partum and then a gradual decline and concluded that stage of lactation had no effect on blood ketones. The present data support a rise to a maximum in early lactation, actually the fifth week post-partum. Although some of the blood ketone levels are high no clinical signs of ketosis were observed. Van Soest et al[104] also observed hyperketonæmia without ketosis. They recorded levels of up to 28 mg./100 ml. of acetone. Thin and Robertson[94] claimed that of the ketone bodies only beta-hydroxybutyric acid was present in the blood of normal cows and that the high blood ketones in ketotic cows were due to an increase in ketone bodies other than the butyrate. In the present data an acetone plus acetoacetic acid fraction is present in all but five of the samples examined and the increased blood ketone levels are due almost entirely to increased levels of beta-hydroxybutyric acid.

The levels of total volatile fatty acids in blood are of the order quoted for normal cows in the literature. McLymont[87] gave a range of 1.3 to 2.1 m.mole/litre in arterial blood of cows on a normal diet as compared with 1.0 to 1.2 m.mole/litre for cows on low roughage diets. Annison et al[147] gave values of 0.71 m.mole/litre for the arterial blood of hay fed sheep and 0.85 m.mole/litre for jugular blood of sheep fed hay and casein. Storry and Rook[148] infused the rumens of cows with various solutions and used a water infusion as a control. They found levels of 0.6 to 1.5 m.mole/litre. The present figures which are stated as a percentage of whole blood fit quite well into this picture except that the figures at the period of maximum concentration are high and are almost comparable with those obtained by Storry and Rook[148] for cows receiving ruminal infusions of acetic acid. The present data on lactational changes in the total volatile fatty acid content of blood cannot be compared with those of other workers but indirect confirmation is offered by Aafjes[100] who noted that blood with a high total ketone content also had a high acetate level. He recorded blood ketone contents of up to 60 mg/100 ml. and blood acetate levels of up to 5 m.mole/litre.

The difference between the composition of the volatile fatty acid fraction of peripheral blood and that which is absorbed from the rumen is striking since propionic and butyric acids have been reduced to insignificant quantities. Sometimes, indeed, they may not even be detectable. The explanation lies in the different metabolic fate of the three acids during and after absorption from the rumen. Barcroft et al[67] showed that the volatile fatty acid content of the blood

draining the rumen was higher than that of peripheral blood and showed the proportion of higher acids to be greater in the rumen than in the blood draining it. Pennington[149] incubated homogenised rumen epithelium with sodium salts of fatty acids and showed butyric acid to be changed to acetone bodies while acetic and propionic acids were little affected. Annison et al[147] examined rumen contents, portal blood and peripheral blood, and showed portal blood to have a lower concentration of butyric acid, relative to acetic and propionic acids, than do rumen contents and that the ketone content of portal blood was higher. Peripheral blood showed a picture very similar to that in the present data. Typical values were acetic acid 92 per cent, propionic acid 3 per cent and butyric acid 0, compared with 75, 21 and 0 in portal blood. McCarthy et al[150] perfused bovine livers with labelled acids and showed that propionic and valeric acids were completely removed by the liver while acetic acid was little affected. Annison and Pennington[79] incubated iso-butyric, iso-valeric, normal-valeric and 2-methylbutyric acids with rumen epithelial slices and found them to be transformed to acetic and propionic acids and ketone bodies. Thus of the acids produced in the rumen practically all except acetic and propionic acids are metabolised in their passage across the rumen epithelium. Propionic acid is removed almost quantitatively in the liver and acetic acid passes almost unchanged to the peripheral circulation.

The data on blood composition show two outstanding phenomena associated with lactation, viz. the occurrence of high levels of acetic acid and beta-hydroxybutyric acids at the third through the fifth week

of lactation. A consideration of the data for rumen contents over the same period shows no significant changes in acetate content. Similarly the slight changes taking place in the beta-hydroxybutyric acid content of the rumen contents bear no relationship to the changes in the blood content. In fact there is a significant ($P = 0.05$) fall in the rumen content of the hydroxybutyrate from week 5 to week 7 but this is too small to affect the blood level. The main source of beta-hydroxybutyric acid in the rumen is normal-butyric acid. There is no significant change in the rumen butyric acid level between stages of lactation and again there is no obvious relationship between the blood constituent and its source material in the rumen. It is reasonable to assume that the changes in blood acetic and beta-hydroxybutyric acids do not occur as a result of ruminal influences.

Comparison of the blood and milk data do indicate certain definite relationships. The period of maximum blood levels of acetic acid and beta-hydroxybutyric acid coincides with the period of maximum production of milk fat, lactose and protein. Since both acids are milk fat precursors it is tempting to speculate that the high levels are present in response to the demands of the mammary gland for fat synthesis. This would involve some controlling mechanism probably hormonal in nature but too little evidence is available for valid discussion of this point. It is interesting, however, to note the observation of Williams et al[151] that treatment of cows with pituitary growth hormone resulted in increased concentrations of plasma free fatty acids and that these have been considered to be a source of endogenous acetate by Annison and White.[152]

A more likely explanation would be that the high acetic and beta-hydroxybutyric acid levels in blood are caused by the blocking of metabolic pathways due to a shortage of some material or materials. Such a shortage could easily arise under the conditions of very high mammary gland activity occurring at this period of lactation. Armstrong[153] has suggested that when the demand for glucose is raised, the activity of enzymes controlling gluconeogenesis is high. As a result there is a shortage of oxalacetate to allow utilisation of acetyl coenzyme A via the tricarboxylic acid cycle. This not only results in an accumulation of acetyl coenzyme A but stimulates fat catabolism to produce the high energy phosphate bonds no longer available from the oxidation of acetyl coenzyme A. Fat catabolism by beta-oxidation produces more acetyl coenzyme A. The resulting build up of the latter will cause it to be metabolised via acetoacetyl coenzyme A to give acetone, acetoacetic acid and beta-hydroxybutyric acid and this would account for a raised blood ketone level. This explanation may also account for the raised blood acetic acid levels since an accumulation of acetyl coenzyme might well cause a shortage of coenzyme A. The theory of oxalacetate deficiency does not explain the observations of Thin et al,[154] Kronfield & Kleiber,[155] and Luick and Smith,[156] that under conditions of fasting and hyperketonæmia, utilisation of acetate for fat production is decreased, which, in itself, would account for the raised blood acetate level. Kronfield and Kleiber[155] suggest that the primary defect causing reduced lipogenesis is a shortage of reduced coenzymes. Such a shortage will exist if the demand for glucose for other purposes is sufficient to

divert supplies from the pentose phosphate pathway of oxidation which is the source of reduced coenzymes in the mammary gland. The reduced lipogenesis occurring under these conditions would in turn reduce the utilisation of acetate and beta-hydroxybutyrate and cause them to accumulate. Under the conditions of very high demand for glucose at the stage of maximum lactose production a shortage of both oxalacetic and reduced coenzymes could exist. There would then be an increased production of ketone bodies and a reduction in utilisation of acetic acid for oxidation. At the same time its utilisation for fat production would be reduced and so would that of beta-hydroxybutyric acid. This explains the increased blood acetic acid and ketone levels and also explains the increased proportion of beta-hydroxybutyric acid in the ketone bodies, as its normal rate of utilisation is reduced. It also offers an explanation of why the fat content of the milk at this period is low when the circulating levels of its main precursors are high.

EXPERIMENT 2

Investigation of the Effect of Calving

EXPERIMENTAL

Animals

Nine Ayrshire cows were used in the experiment. Details are given in Table 6.

Table 6

Details of Cows used in Experiment 2

Cow	Age Years	Lactation	Live Weight lb.
7	8	6	1095
8	7	4	1160
9	7	5	995
10	6	3	1325
11	8	5	1170
12	8	5	895
13	11	7	1160
14	10	6	865
15	7	5	1220

Feeding and Management

The cows were housed in an ordinary byre from about two months before calving to the end of experiment on the sixteenth day post-partum. All animals were given a basic ration of sixteen pounds of hay throughout the experimental period. The composition and nutritive value of the hay are given in Table 7.

Table 7

Composition and Nutritive Value of Hay fed in Experiment 2

	per cent
Dry Matter	83.3
Crude Protein	7.4
Crude Fibre	25.0
Calcium	0.29
Phosphorus	0.27
Magnesium	0.11
Estimated DCP	3.69
Estimated SE	33

Estimates of DCP and SE were made using the regression equations of Watson and Nash.[131]

At the beginning of the experimental period the cows were given about two pounds of a concentrate mix per day in addition to the hay ration. The concentrate mix was made up of seven parts of barley, five parts of oats, five parts of flaked maize, one and a half parts of groundnut cake and one and a half parts of soyabean meal. Thirty five pounds of ground limestone and two pounds of calcined magnesite were added per ton of the mix. The composition and nutritive value of the concentrate mix is given in Table 8.

Table 8

Composition and Nutritive Value of Concentrate Mix fed in Experiment 2

	per cent
Dry Matter	87.2
Crude Protein	16.0
Ether Extract	3.4
Crude Fibre	5.5
Nitrogen-free-extractives	60.5
Ash	2.8
Calcium	0.80
Phosphorus	0.56
Magnesium	0.19
Estimated DCP	12.8
Estimated SE	71

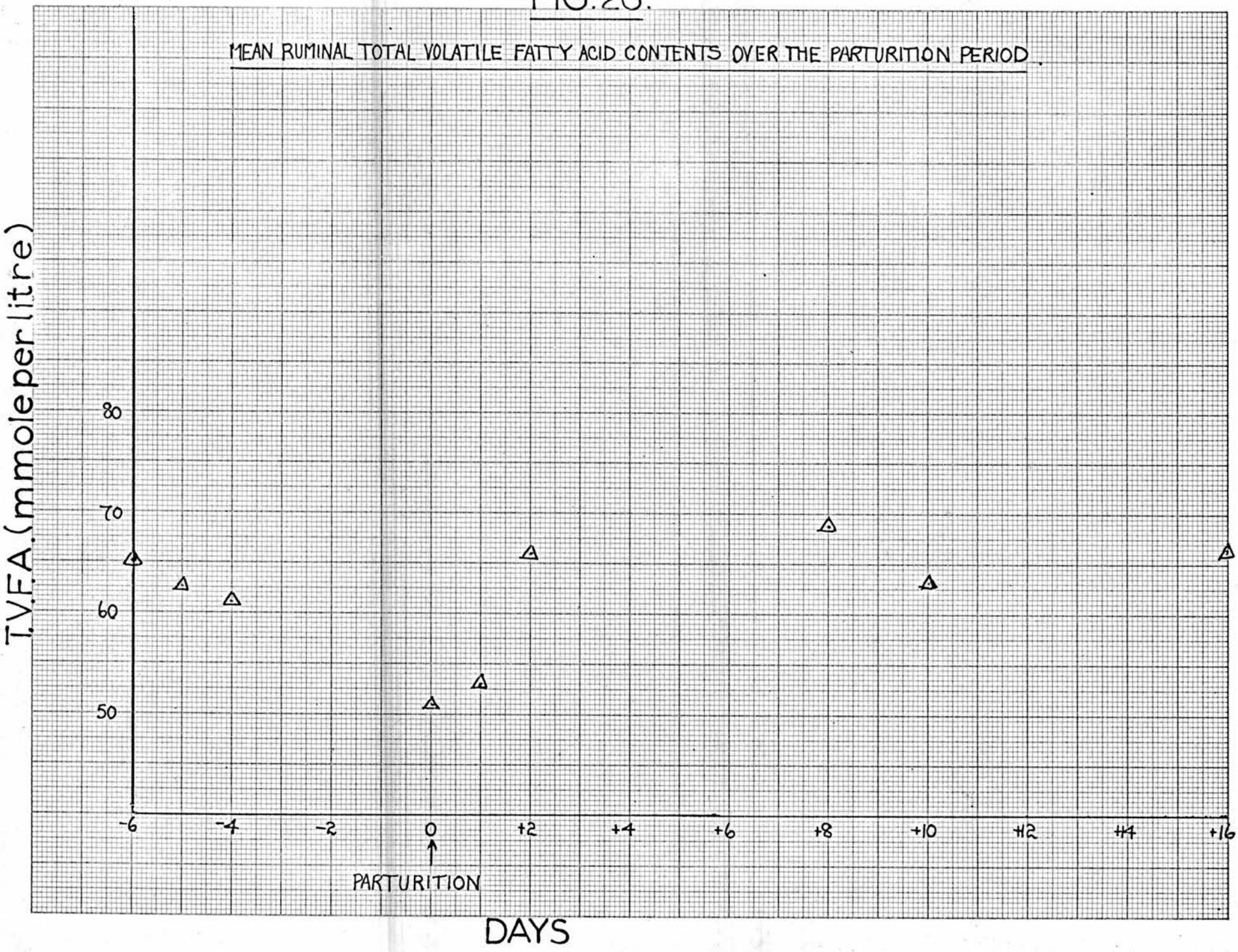
The allowance of concentrate was increased gradually during the period before calving so that six to eight pounds per day were being fed at calving. After calving no concentrates were offered but hay was available. On the day following calving four pounds of concentrates were offered in two feeds and the allowance built up rapidly to full feed at two weeks post-partum. The allowance was 110 per cent of the requirement based on yield, at four pounds per gallon. Concentrates

were given twice daily at 6.0 a.m. and 3.30 p.m. Hay was fed at 11.0 a.m. and 5.0 p.m. There were considerable differences in feeding behaviour between the cows over the period of calving. Cow No. 7 ate very little food for two weeks post-partum consuming only about six pounds of hay daily and no concentrate for the first seven days. From this point on the concentrate intake increased gradually but by the end of the experiment concentrate intake was still only four to five pounds per day. Cows 11, 13 and 14 showed hardly any check in their feed intake at all. They consumed the hay provided after parturition and in the day following consumed the concentrates offered. By the end of the experimental period they were being fed according to the allowance previously described. Cows 8, 9, 10 and 12 showed a much reduced intake of hay following calving but on the day following consumed their ration of hay with about one and a half pounds of concentrate. On the next day they consumed the hay plus four pounds of concentrate. By the end of the experiment they were being fed their full allowance according to yield.

Cow No. 9 escaped on the eighth day before calving and gorged herself on bruised barley. On the seventh and sixth day before calving she scoured very badly. She refused food on the seventh day and took a very little hay on the sixth day. On the fifth day before calving she consumed her ration of hay and a little concentrate and was back to normal intakes two days later.

Samples of blood and rumen contents were taken on three consecutive days in the week preceding calving to establish a pre-partum standard with which to compare other samples taken during the

FIG. 26.


MEAN RUMINAL TOTAL VOLATILE FATTY ACID CONTENTS OVER THE PARTURITION PERIOD.
T.V.F.A. (mmole per litre)
80
70
60
50
-6
-4
-2
0
+2
+4
+6
+8
+10
+12
+14
+16
PARTURITION
DAYS

investigation. These samples are described as having been taken on the sixth, fifth and fourth days prior to calving. Although not strictly true this is considered justifiable from the point of view of convenience, and, as the samples are not meant to represent strict time intervals no misrepresentation is involved. Samples were taken during the twenty four hours following calving. The time interval between calving and sampling was at least six hours and in some cases was as much as eighteen hours. Subsequently samples were taken on the first, second, eighth, tenth and sixteenth days post-partum. Sampling was carried out at the standard three hours after the morning concentrate feed as described previously.

RESULTS

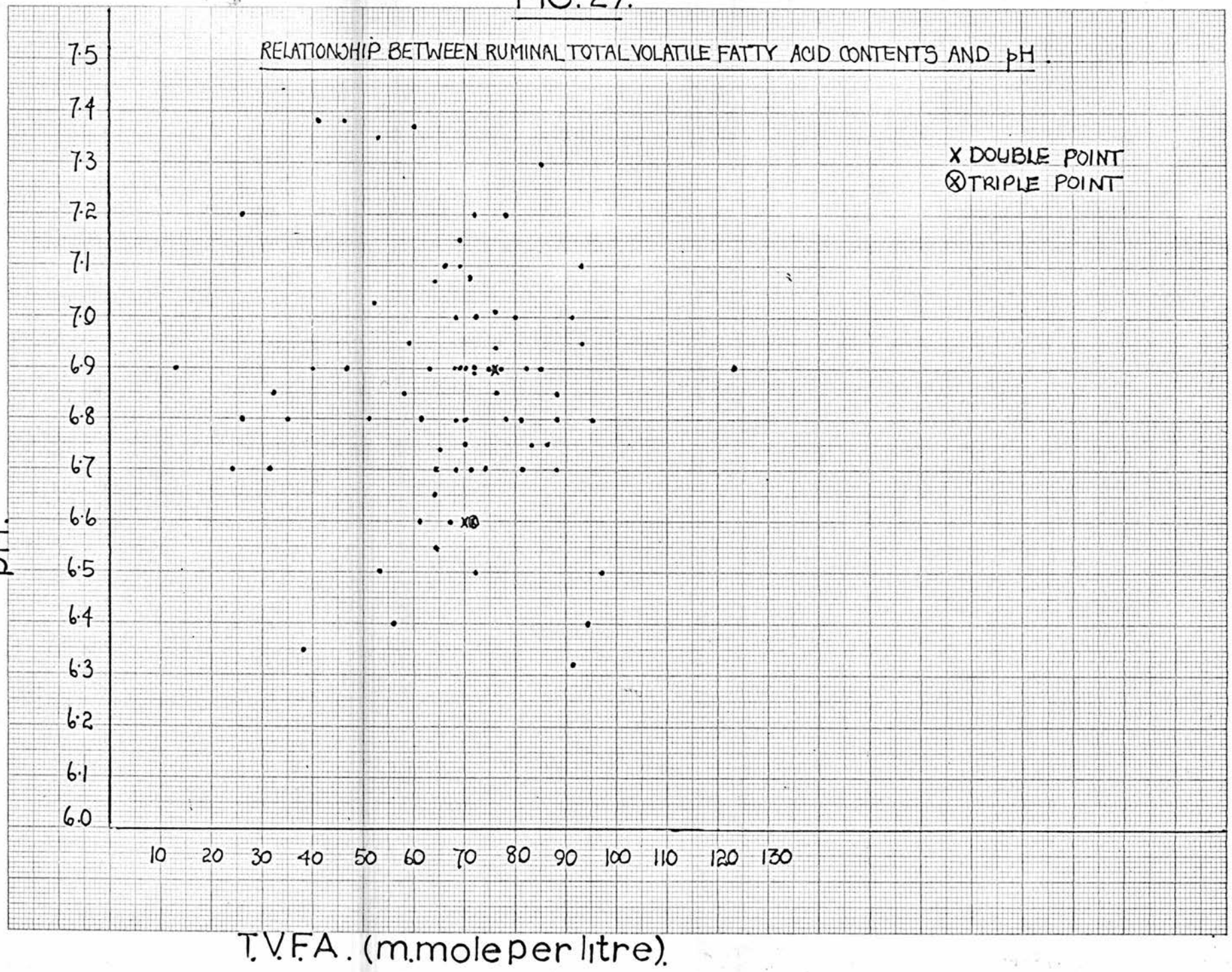
Rumen Contents

The analytical results for the samples of rumen contents are given in Appendix Tables 25, 28, 31, 34, 37, 40, 43, 46 and 49.

pH The range of pH values recorded was 6.35 to 7.38 with a mean of 6.84. An analysis of variance gave no evidence for differences between cows or between stages.

Total Volatile Fatty Acids The range of values was 12.9 to 122.6 m.mole/litre with a mean of 67.7 m.mole/litre. An analysis of variance gave significant evidence for differences between cows ($P = 0.001$) but none for differences between stages. The mean total volatile fatty acid contents for the nine cows at different stages are shown in Fig. 26. Despite the absence of statistically significant differences between stages there does seem to be a definite lowering of total volatile fatty acid content on

FIG. 27.

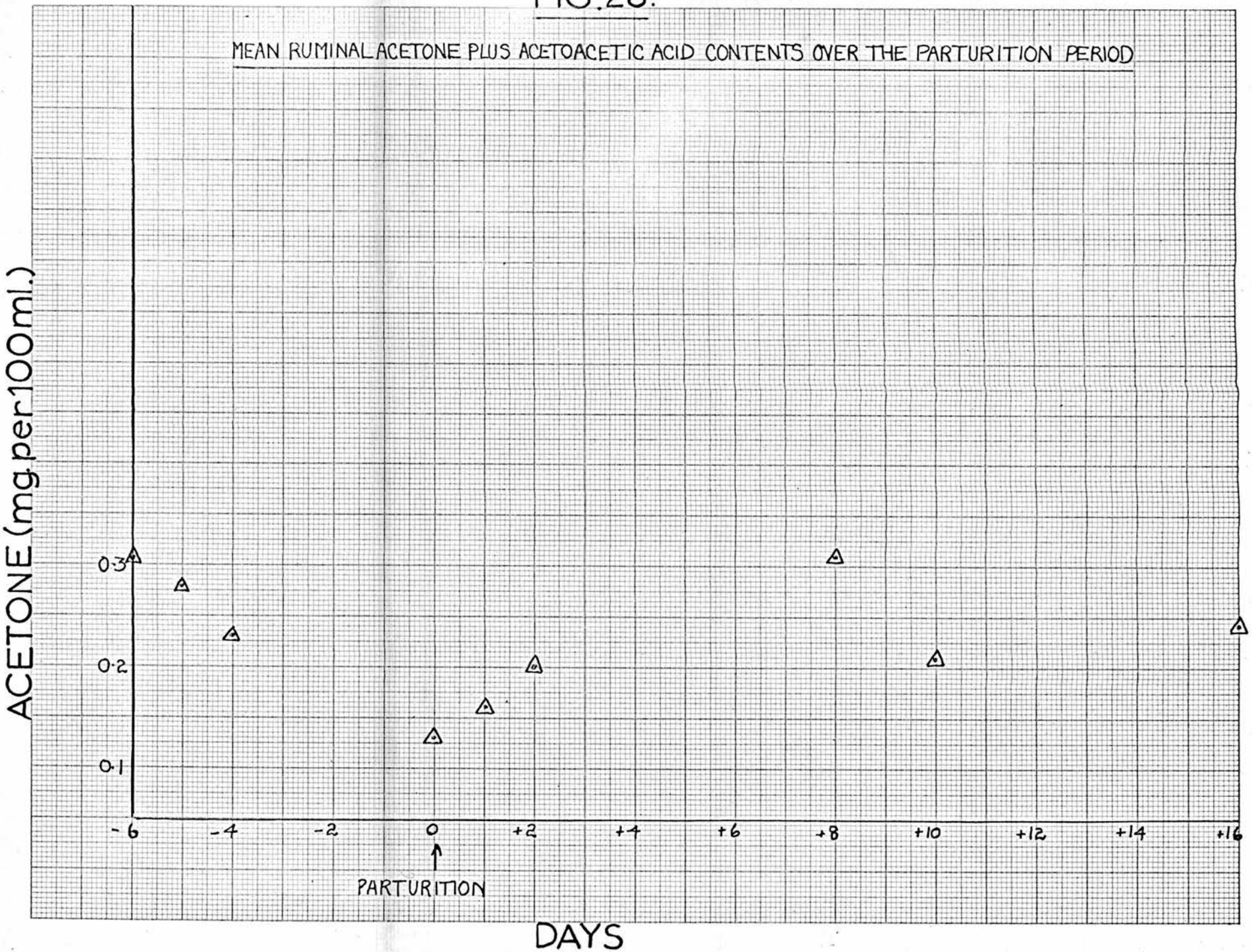

RELATIONSHIP BETWEEN RUMINAL TOTAL VOLATILE FATTY ACID CONTENTS AND pH.
X DOUBLE POINT
⊗ TRIPLE POINT
pH.
7·5
7·4
7·3
7·2
7·1
7·0
6·9
6·8
6·7
6·6
6·5
6·4
6·3
6·2
6·1
6·0
10
20
30
40
50
60
70
80
90
100
110
120
130
T.V.F.A. (m.mole per litre).

the day of parturition and that immediately following. Examination of the results for individual cows shows that six out of the nine animals showed lower values at either stage four or five than in the pre-partum period. There are striking differences in the data for different cows. Cow No. 7 showed a low total volatile fatty acid content from the beginning but this was particularly noticeable after calving and normal levels were not achieved until the sixteenth day post-partum. Cows Nos. 8, 10, 12 and 15 had low values at the fourth and fifth stage. Cows Nos. 11, 13 and 14 showed little or no change throughout the experimental period. Cow No. 9 had a low ruminal total volatile fatty acid content at the second, third, fourth and fifth stage. The relationship between pH and total volatile fatty acid content is shown in Fig. 27. There is no correlation between the two.

Individual Volatile Fatty Acids Acetic, propionic, butyric, iso-butyric, iso-valeric and n-valeric acids were present in all the rumen contents. Traces of caproic acid were found in the liquors of all cows on occasions. The amounts were not measurable and have not been included in the appendix tables. Analyses of variance gave significant evidence for differences between cows in acetic acid content ($P = 0.001$), propionic acid content ($P = 0.05$), iso-butyric acid ($P = 0.01$) and valeric acid content ($P = 0.05$), and differences between stages in propionic and valeric acids ($P = 0.05$). On the whole differences in the amounts of the individual fatty acids reflect the differences in total volatile fatty acid content.

The proportions of the individual acids stated as molar

FIG. 28.


MEAN RUMINAL ACETONE PLUS ACETOACETIC ACID CONTENTS OVER THE PARTURITION PERIOD
ACETONE (mg. per 100 ml.)
0·3
0·2
0·1
-6
-4
-2
0
+2
+4
+6
+8
+10
+12
+14
+16
PARTURITION
DAYS

percentages of the total volatile acid content are given in Appendix Tables 26, 29, 32, 35, 38, 41, 44, 47 and 50. Analyses of variance gave significant evidence for differences between cows in acetic acid and propionic acid (P = 0.001) and iso-butyric acid, iso-valeric acid and valeric acid (P = 0.01). Only for valeric acid was there evidence for differences between stages. The molar proportion of valeric acid at stage four is significantly higher than any other except stage seven which is significantly higher than stages one, two, three and six. Examination of the data for individual cows shows considerable differences but there is some evidence of an inverse relationship between propionic and acetic acids. The mean values for the amounts and proportions of the individual acids are given in Table 9.

Table 9.

Mean Concentrations and Molar Percentages of Individual Volatile Fatty Acids in Rumen Liquor

	Molar percentage	m.mole/litre
Acetic Acid	67.0	45.6
Propionic Acid	15.7	10.5
Butyric Acid	13.1	8.8
Iso-butyric Acid	1.3	0.8
Iso-valeric Acid	1.7	1.1
Valeric Acid	1.2	0.8

Acetone + Acetoacetic Acid This fraction was present in all but four of the samples of rumen liquor analysed. The range of values extended from 0 to 0.80 mg./100 ml., as acetone, with a mean of 0.23 mg./100 ml. An analysis of variance gave significant evidence of differences between cows (P = 0.001) but not between stages. Mean values for the nine cows at different stages are given in Fig. 28. The values at stages four and five are lower

FIG. 29.

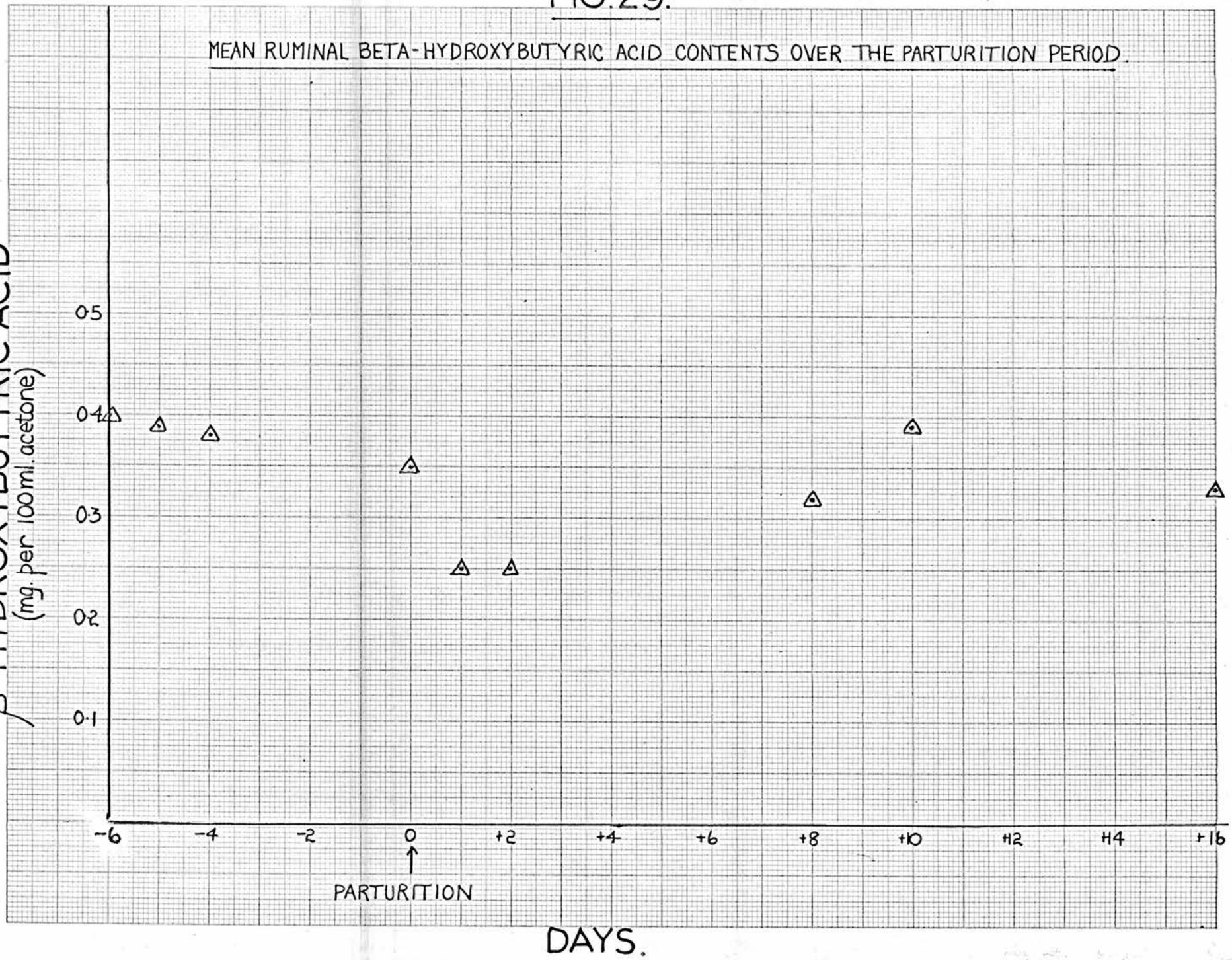

MEAN RUMINAL BETA-HYDROXYBUTYRIC ACID CONTENTS OVER THE PARTURITION PERIOD.
B-HYDROXYBUTYRIC ACID
(mg. per 100 ml. acetone)
0·5
0·4
0·3
0·2
0·1
-6
-4
-2
0
+2
+4
+6
+8
+10
+12
+14
+16
PARTURITION
DAYS.

FIG.30.

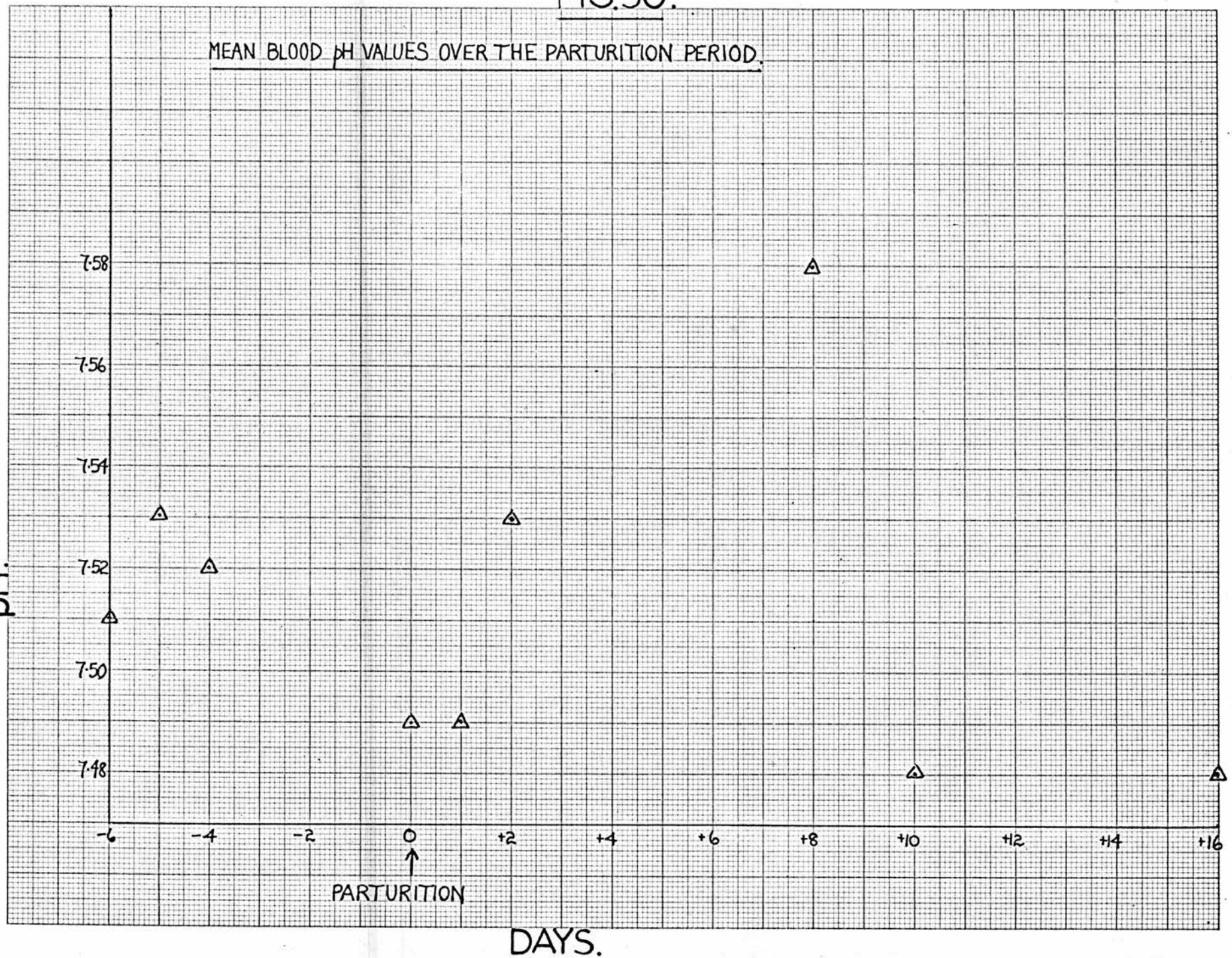
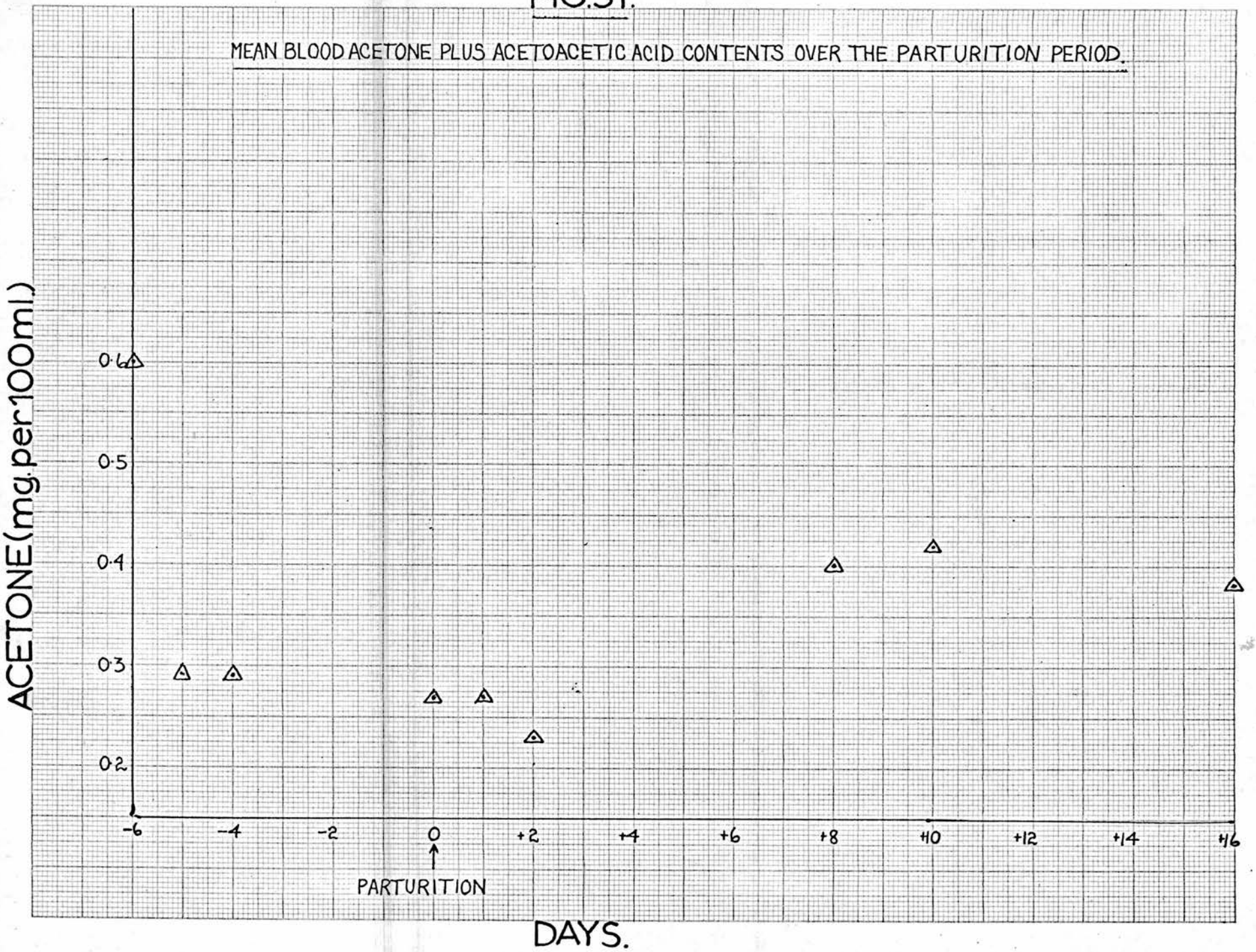

MEAN BLOOD pH VALUES OVER THE PARTURITION PERIOD.
7·58
7·56
7·54
7·52
7·50
7·48
pH.
-6
-4
-2
0
+2
+4
+6
+8
+10
+12
+14
+16
PARTURITION
DAYS.

FIG.31.


MEAN BLOOD ACETONE PLUS ACETOACETIC ACID CONTENTS OVER THE PARTURITION PERIOD.
ACETONE (mg. per 100 ml.)
0·6
0·5
0·4
0·3
0·2
-6
-4
-2
0
+2
+4
+6
+8
+10
+12
+14
+16
PARTURITION
DAYS.

than at the others. Examination of the data for individual cows shows that values at these two stages are lower than the prepartum values for seven out of the nine cows.

Beta-hydroxybutyric Acid The range of values is 0 to 1.36 mg./100 ml. as acetone. An analysis of variance gave no significant evidence of differences between cows or stages. Fig. 29 shows the mean values for beta-hydroxybutyric acid content for the nine cows at different stages in the experiment. Stages five and six show lower values than other stages. Both stages are lower than the general pre-partum level for seven out of nine cows.

Blood

The results of the analyses carried out on the blood samples collected during the experiment are given in Appendix Tables 24, 27, 30, 33, 36, 39, 42, 45 and 48.

pH The range of pH values was from 7.38 to 7.72 with a mean of 7.51. An analysis of variance gave no significant evidence of differences between cows or between stages. The mean values at different stages are given in Fig. 30.

Acetone + Acetoacetic Acid The range of values was from 0 to 1.30 mg./100 ml. as acetone with a mean of 0.35 mg./100 ml. The fraction was present in all but three samples. An analysis of variance gave significant evidence of differences between cows ($P = 0.001$) and between stages ($P = 0.01$). The mean values for the nine cows at different stages are plotted in Fig. 31. There is a drop from the first stage to a low value which is maintained to stage seven when the value rises again. Only stage one is

FIG. 32

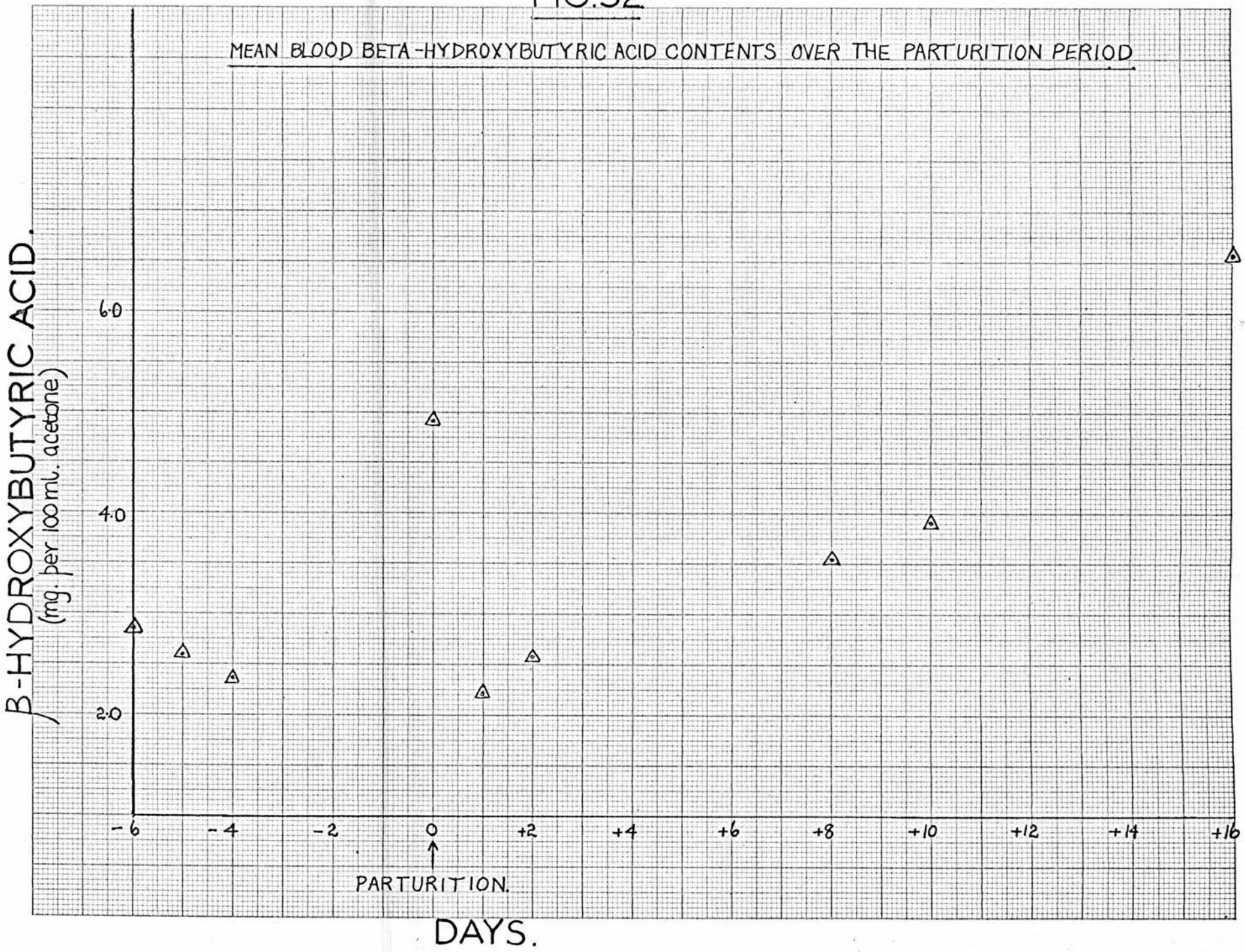
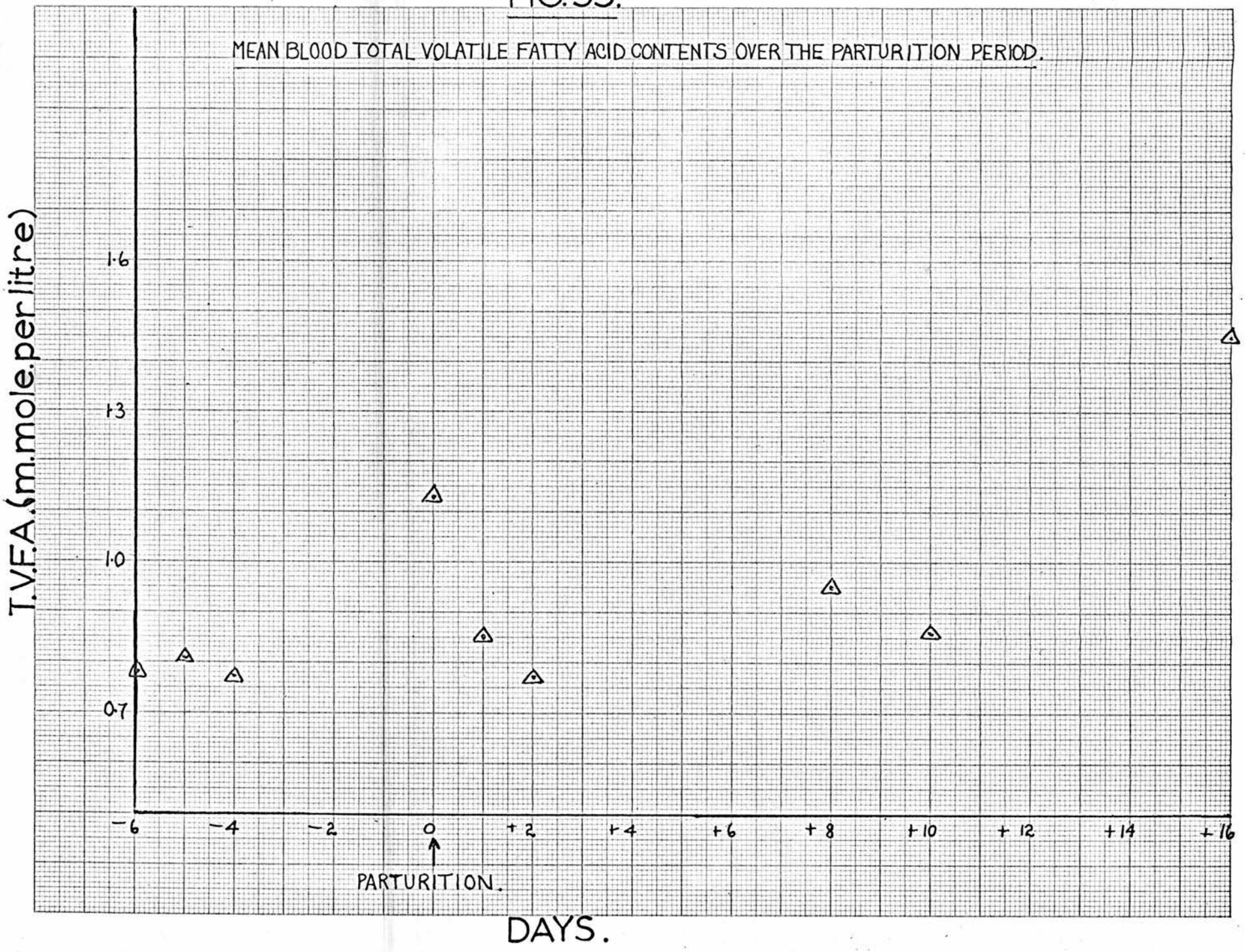

MEAN BLOOD BETA-HYDROXYBUTYRIC ACID CONTENTS OVER THE PARTURITION PERIOD
B-HYDROXYBUTYRIC ACID.
(mg. per 100 ml. acetone)
6·0
4·0
2·0
-6
-4
-2
0
+2
+4
+6
+8
+10
+12
+14
+16
PARTURITION.
DAYS.

FIG. 33.

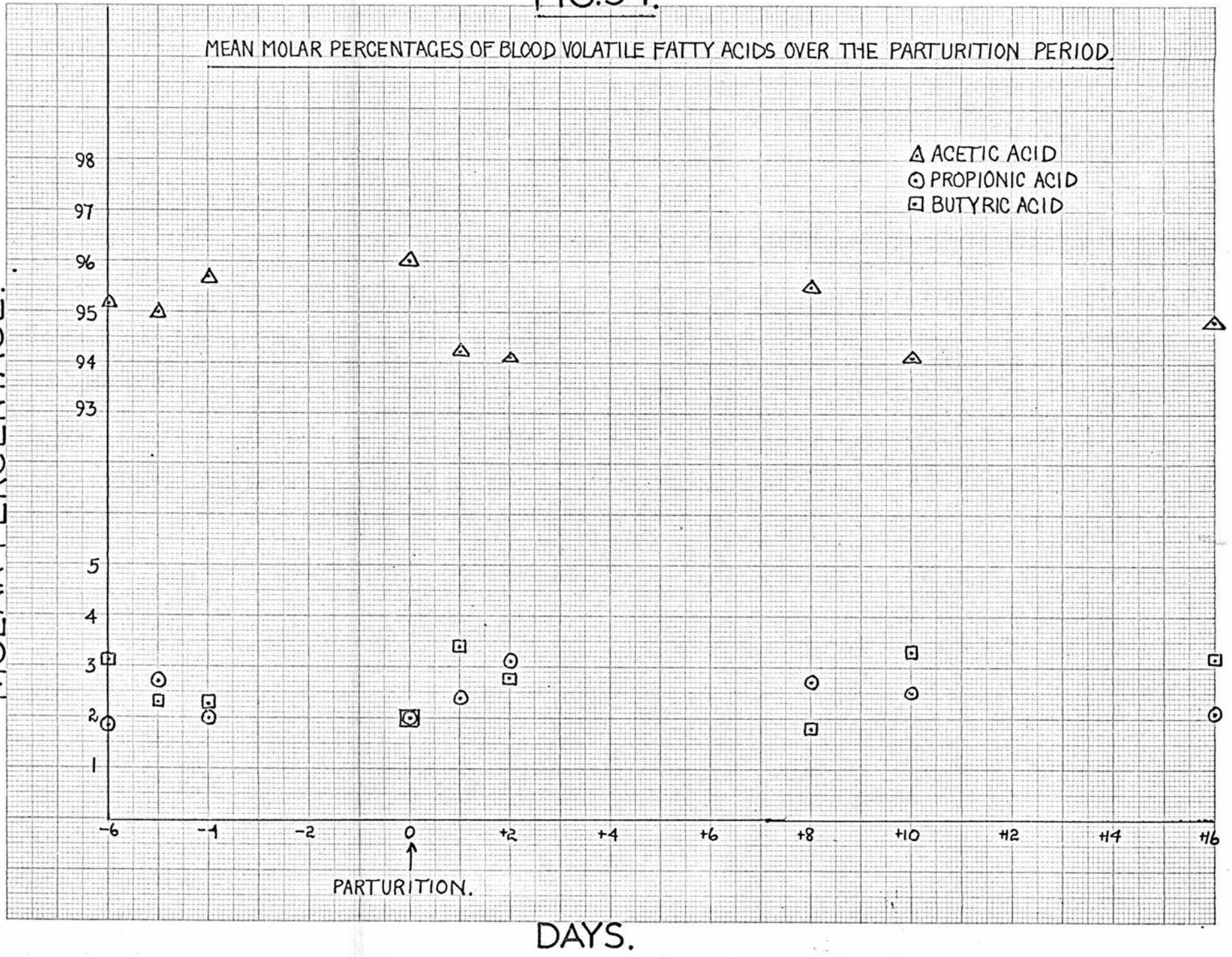

MEAN BLOOD TOTAL VOLATILE FATTY ACID CONTENTS OVER THE PARTURITION PERIOD.
T.V.F.A. (m.mole per litre)
1·6
1·3
1·0
0·7
−6
−4
−2
0
+2
+4
+6
+8
+10
+12
+14
+16
PARTURITION.
DAYS.

significantly different from the others.

Beta-hydroxybutyric Acid A range of beta-hydroxybutyric acid values from 0.78 to 10.23 mg./100 ml., as acetone, was recorded. The mean value was 3.51 mg./100 ml. An analysis of variance gave significant evidence for differences between cows ($P = 0.01$) and between stages ($P = 0.001$). The mean blood beta-hydroxybutyric acid contents for nine cows at different stages are plotted in Fig. 32. There is a sharp rise at stage four but the value drops back to the prepartum level at stage five. There is then a gradual rise to the end of the experimental period at sixteen days post-partum. The rise at stage four is statistically significant and values at stages seven, eight and nine are also significantly higher than at other stages.

Total Volatile Fatty Acids The values for the total volatile fatty acid contents of the blood samples cover a range from 0.43 to 1.95 m.mole/litre with a mean of 0.93 m.mole/litre. An analysis of variance gave significant evidence ($P = 0.001$) for differences between cows and between stages. The mean figures for the nine cows at different stages are plotted in Fig. 33. There is a sharp rise at stage four but a drop back to the prepartum level at stage six. From this point the blood volatile fatty acid content rises to the end of the experimental period. The rise at stage four is statistically significant and the value at stage nine is significantly higher than at other stages and that at stage seven is higher than one, three and six.

FIG.34.


MEAN MOLAR PERCENTAGES OF BLOOD VOLATILE FATTY ACIDS OVER THE PARTURITION PERIOD.
ACETIC ACID
PROPIONIC ACID
BUTYRIC ACID
MOLAR PERCENTAGE.
98
97
96
95
94
93
5
4
3
2
1
-6
-1
-2
0
+2
+4
+6
+8
+10
+12
+14
+16
PARTURITION.
DAYS.

Individual Volatile Fatty Acids The figures in the appendix tables are given as molar percentages as in the previous experiment. Analyses of variance showed significant evidence for differences between cows for all acids ($P = 0.01$) but none for differences between stages. The mean molar percentages at different stages are shown in Fig. 34. There appears to be a slight increase in acetic acid and a decrease in propionic and butyric acids at stage four.

DISCUSSION

The range of ruminal total volatile fatty acid contents in the present data is very wide and is characterised by the relatively large number of samples having low values. That of 12.9 m.mole/litre for Cow No. 9 on the fourth day pre-partum is the lowest recorded by the author and the only comparable figures in the literature are 38 m.mole/litre quoted by Coop[138] and 49 m.mole/litre given by Gray and Pilgrim.[75] Coop obtained his low value with sheep after a fast of about thirty six hours and this compared with normal values on pasture of 160 m.mole/litre. Gray and Pilgrim fasted sheep for about seventeen hours and took samples of rumen contents after feeding. Low values were obtained with samples taken at "zero" time after feeding which, as well as low total volatile fatty acid content, showed a higher proportion of acetic acid and a lower proportion of propionic acid than those from fully fed animals. The proportion of butyric acid showed no change. Schultz[72] starved cows for about sixteen hours and obtained ruminal total volatile fatty acid contents of 65.4 m.mole/litre compared with 102.8 m.mole/litre for fully fed animals. He could

show no differences in the proportions of the major volatile acids. Brown and Shaw[74] starved cows for eighteen to twenty four hours and found levels of total volatile fatty acids of 51.3 m.mole/litre compared with 88.8 m.mole/litre for control cows. The proportion of acetic acid increased and that of propionic acid decreased in the rumen liquors of fasted cows. The rumen liquor giving the low value of 12.9 m.mole of total volatile fatty acids per litre exhibits other characteristics typical of those from fasted animals in that the molar percentage of acetic acid was 85.6 and of propionic acid 9.1. In addition the proportion of butyric acid was much reduced at 3.0 per cent. Although Cow No. 9 had been subjected to a fast and reduced intake before the sample was taken, it had also suffered a considerable digestive upset and there seems little doubt that this contributed to the production of the very abnormal rumen conditions of which the low value is indicative. Coop[138] has claimed that there is a considerable emptying of the rumen when animals are starved and that under such conditions microfloral activity is reduced. The scouring in Cow No. 9 together with the reduced intake could have had this effect and resulted in a low ruminal total volatile fatty acid content.

Fasting probably accounts for the low ruminal total volatile fatty acid contents shown by some of the cows on the day of parturition and that following. These cows were those which have already been described as having reduced intakes of food at this time. At least six hours elapsed between calving and sampling and in some cases the interval was as much as eighteen hours. This argues a period of fast or reduced intake for anything up to twenty

hours before sampling immediately post-partum (stage 4). This was followed by twenty four hours of reduced intake before the next sample (stage 5) was taken. It is not surprising that such samples showed low total volatile fatty acid contents. Animals were eating almost normally before the next sample was taken at stage 6 when the composition showed a return to normality. This agrees with the contention of Coop[138] that return to normal after the end of a fast takes about eighteen hours. Three of the cows showed little or no change in food intake over the parturition period and as would be expected showed little or no change in rumen contents. One cow, No. 7, showed very much reduced intakes after calving and these are reflected in the generally low level of total volatile fatty acids in rumen samples taken from this cow, until near the end of the experimental period. The composition of the volatile acid fraction in these rumen samples is certainly not typical of fasted animals showing molar percentages of acetic acid of 47.9 and 52.8 accompanied by 34.2 and 26.9 per cent for propionic acid on two occasions and only on the day of parturition and that following were molar percentages of propionic acid below the general mean for the data. Examination of the data for individual cows with regard to molar proportions of the major acids in rumen liquor samples show a confused and contradictory picture. Table 10 shows the total volatile fatty acid content and molar proportions of acids in rumen samples taken on the day of parturition and that following for Cow No. 12 which showed low total volatile fatty acid contents and Cow No. 13 which showed normal levels.

Table 10

Composition of Rumen Samples at Parturition

	Total Volatile Fatty Acids (m.mole/litre)	Acetic Acid (Molar per cent)	Propionic Acid (Molar per cent)	Butyric Acid (Molar per cent)
Cow 12 Stage 4	40.6	73.25	10.00	10.25
Cow 12 Stage 5	59.8	55.18	19.23	19.57
Cow 13 Stage 4	84.7	73.79	14.17	9.68
Cow 13 Stage 5	75.6	62.47	14.99	17.64

On balance it would probably be true to say that the present data give some support to the view that the reduced total volatile fatty acid contents resulting from fasting contain higher acetic and lower propionic acid contents than normal.

Ruminal acetone + acetoacetic acid levels in the present instance are much lower than those reported in Experiment 1 and the mean value is only about a tenth of that in the latter. The explanation is not obvious. It may be that fasting lowers the level of this fraction as well as the volatile acids. Beta-hydroxybutyric acid contents are also lower than in Experiment 1 but the discrepancy is not great and values are of a like order i.e. a mean of 0.34 compared with 0.53 mg./100 ml.

Fig. 28 shows no significant change in blood acetone + acetoacetic acid over the parturition period. The beta-hydroxybutyric acid contents of the blood samples, on the other hand, show a well defined pattern over the experimental period with a rise on the day of parturition, which is significant but temporary, and a return to normal pre-partum levels the following day. There is then a rise as lactation proceeds. This latter effect has already been noted in

the first experiment and has been discussed in considerable detail. As before, the rise in beta-hydroxybutyric acid is accompanied by a rise in blood acetic acid. The rise in beta-hydroxybutyric acid on the day of parturition is not easily explained in the same terms since a shortage of glucose arising and ceasing so quickly is difficult to envisage. Furthermore, Van Soest and Blosser[117] and Merril and Smith[118] have shown blood glucose levels to be high immediately before and after parturition. The high circulating glucose levels argue mobilisation and this is borne out by the work of Merril and Smith[118] who showed increased ACTH secretion as a result of stress at parturition. This would stimulate glucocorticoid secretion by the adrenal cortex and cause glucose mobilisation and result, in turn, in a depletion of liver glycogen. If this were low originally a shortage could result and fat catabolism be initiated with consequent production of ketone bodies. The high circulating glucose levels could reduce utilisation of ketones and acetic acid for energy purposes and cause them to accumulate. With the passing of the stress effect, conditions return to normal, blood glucose levels fall,[118] and any accumulated acetic acid and beta-hydroxybutyric acid would be used in milk production and blood levels return to normal. Shaw[96] showed high blood levels of ketones in cows on the day of parturition and suggested that this might be due to lowered liver glycogen. He noted that adrenalin administration depleted liver glycogen and caused a rise in blood glucose. The secretion of adrenalin owing to the stress of parturition could account for the high blood levels of beta-hydroxybutyric acid and acetic acid at this time.

EXPERIMENT 3

Investigation of the Effects of Cessation of Milking

EXPERIMENTAL

Animals

Seven Ayrshire cows were used in the experiment. Details are given in Table 11.

Table 11

Details of Cows used in Experiment 3

Cow	Age Years	Lactation	Live Weight lb.	Yield at Drying Off lb.
16	5	3	1060	12
17	5	3	1150	9
18	5	3	1035	12
19	6	4	1204	15
20	6	4	950	14
21	6	4	950	21
22	5	2	975	10

Feeding and Management

Cows were selected four weeks before they were to be dried off and remained on the experiment until the seventeenth day after drying-off. Throughout the period the animals were fed a daily ration of sixteen pounds of hay plus enough concentrates to allow for the production of one gallon of milk. The composition and nutritive value of the hay is given in Table 12.

Table 12

Composition and Nutritive Value of Hay fed in Experiment 3

	per cent
Dry Matter	84.8
Crude Protein	6.8
Crude Fibre	28.3
Calcium	0.37
Phosphorus	0.20
Magnesium	0.09
Estimated DCP	3.2
Estimated SE	29.5

Estimates of DCP and SE were made using the regression equations of Watson and Nash.[131] The concentrate was the same as that used in Experiment 2 and had the composition and nutritive value shown in Table 8. Hay was fed daily at 11.0 a.m. and 5.0 p.m. and concentrates at 6.0 a.m. and 3.30 p.m. Cows were dried off by ceasing to milk them on the day chosen for drying off. They were examined daily at subsequent morning and evening milkings and when necessary milk was withdrawn from the udder to relieve pressure. This was done on day three after drying off for cows 17, 18 and 19 and on day four after drying off for cows 16, 20, 21 and 22. No further withdrawals of milk were necessary and the daily examination was discontinued after one week. Drying-off had no obvious effects on the animals and throughout the experimental period they consumed their full ration of food.

Samples of blood and rumen contents were taken four, three and two days prior to drying-off and on days one, three, five, nine, eleven and seventeen after drying off. Sampling was carried out about three hours after the morning concentrate feed as previously described.

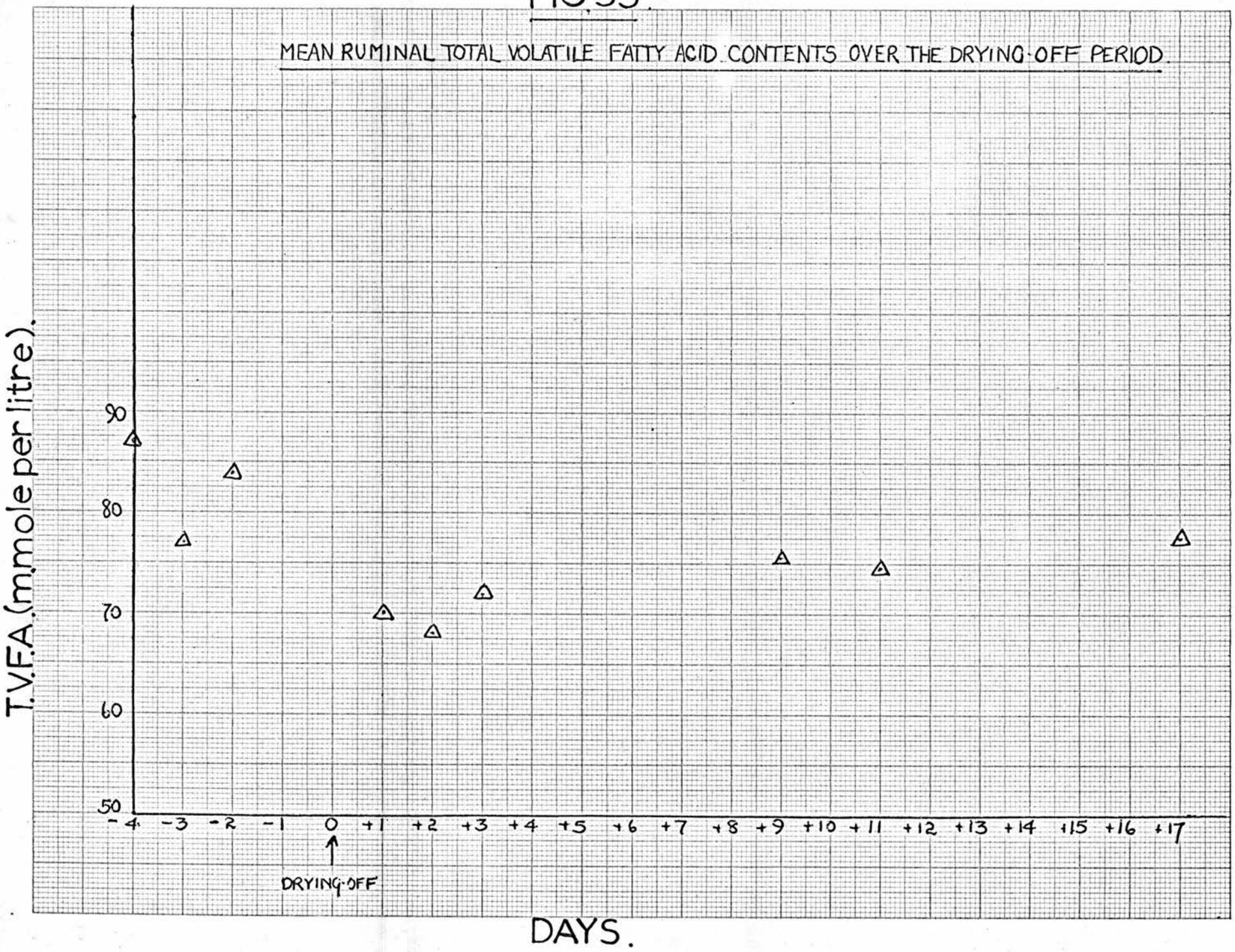
RESULTS

Rumen Contents

The analytical results for the samples of rumen contents are given in Appendix Tables 51, 54, 57, 60, 63, 66 and 69.

pH The range of pH values recorded was from 6.0 to 7.4 with a mean of 6.77. An analysis of variance did not

FIG 35.


MEAN RUMINAL TOTAL VOLATILE FATTY ACID CONTENTS OVER THE DRYING-OFF PERIOD.
T.V.F.A. (m.mole per litre).
90
80
70
60
50
-4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8 +9 +10 +11 +12 +13 +14 +15 +16 +17
DRYING-OFF
DAYS.

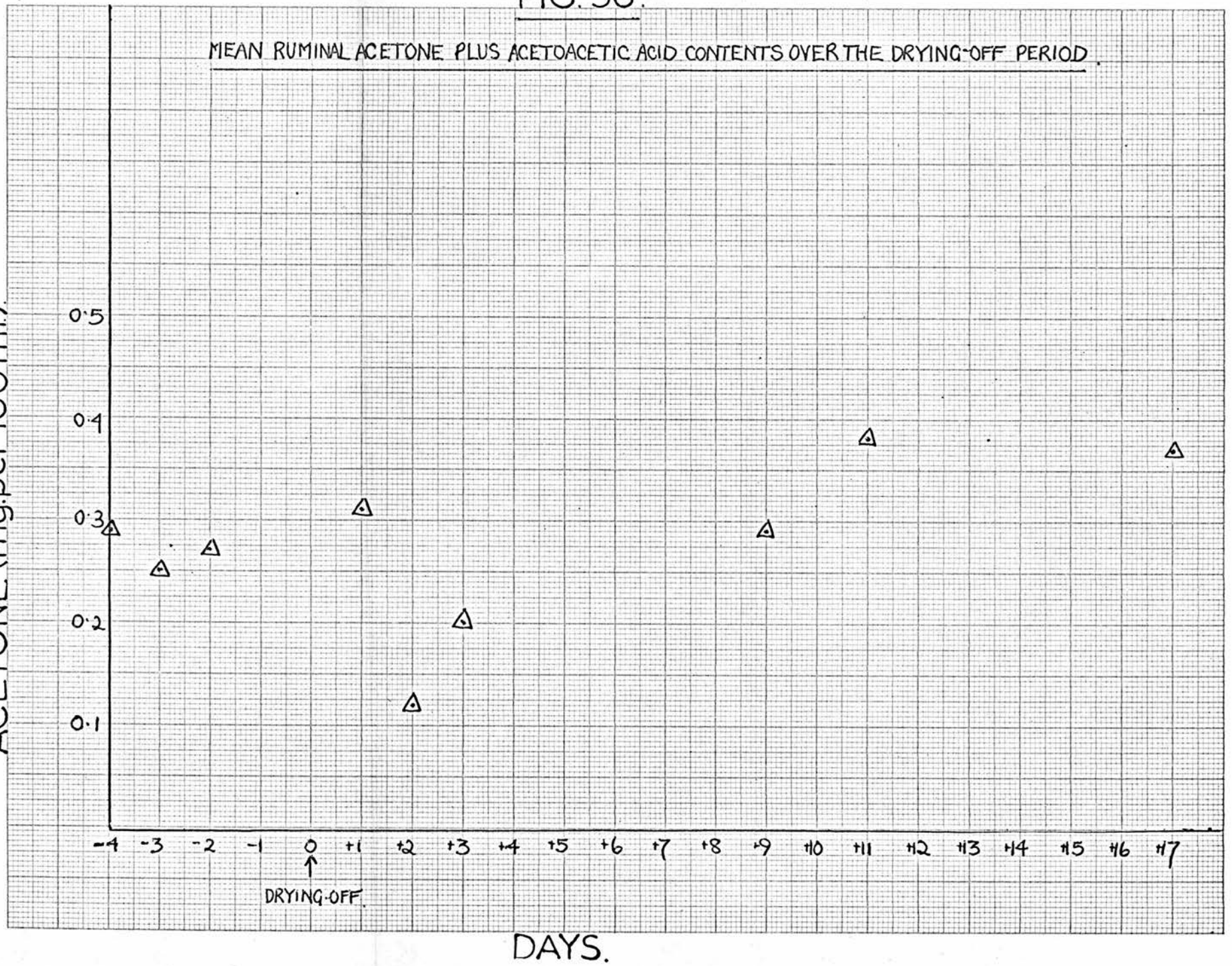
give significant evidence of differences between cows or between stages.

Total Volatile Fatty Acids Recorded values ranged from 42.6 to 101.5 m.mole/litre with a mean of 76.1 m.mole/litre. An analysis of variance gave significant evidence ($P = 0.01$) for differences between stages but not between cows. Mean total volatile fatty acid contents for the seven cows are shown in Fig. 35. Values are lower at the three stages following upon drying off but there is no significant difference ($P = 0.05$) between them and those at other stages except for one and three. There is a negative correlation between total volatile fatty acid content and pH ($r = -0.38$). Although significant ($P = 0.01$) the correlation is not close since only about fourteen per cent of the variation in pH is accounted for by variation in total volatile fatty acid content.

Individual Volatile Fatty Acids Acetic, propionic, butyric, iso-butyric, iso-valeric and valeric acids were present in all the samples of rumen contents. Traces of caproic acid were found in the liquors of all cows on a number of occasions. The amounts were not measurable and have not been included in the appendix tables. Analyses of variance gave significant evidence for differences between cows in the content of acetic and butyric acids ($P = 0.01$), propionic acid ($P = 0.05$) and iso-valeric and valeric acids ($P = 0.001$). There was significant evidence for differences between stages in the contents of acetic acid ($P = 0.01$), butyric acid ($P = 0.001$), and iso-valeric acid ($P = 0.05$).

The proportions of the individual acids stated as molar

FIG. 36.


MEAN RUMINAL ACETONE PLUS ACETOACETIC ACID CONTENTS OVER THE DRYING-OFF PERIOD.
ACETONE (mg. per 100 ml.)
0·5
0·4
0·3
0·2
0·1
-4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8 +9 +10 +11 +12 +13 +14 +15 +16 +17
DRYING-OFF
DAYS.

percentages of the total volatile fatty acid fraction are given in Appendix Tables 53, 56, 59, 62, 68 and 71. Analyses of variance gave significant evidence for differences between cows in the contents of acetic, butyric, iso-valeric and valeric acids (P = 0.001) and propionic acid (P = 0.01). Only in the case of iso-valeric acid (P = 0.05) was there significant evidence for differences between stages. Mean values for the amounts and proportions of the individual acids are given in Table 13.

Table 13

Mean Concentrations and Molar Percentages of Individual Volatile Fatty Acids in Rumen Liquor

	Molar Percentage	m.mole/litre
Acetic Acid	70.8	53.9
Propionic Acid	13.5	10.3
Butyric Acid	12.3	9.3
Iso-butyric Acid	1.0	0.8
Iso-valeric Acid	1.5	1.1
Valeric Acid	0.9	0.7

Acetone + Acetoacetic Acid This fraction was present in all samples of rumen contents analysed. The range of values was 0.05 to 0.71 mg./100ml., as acetone, with a mean value of 0.27 mg./100 ml. An analysis of variance gave significant evidence of differences between stages (P = 0.001) but not between cows. Mean values for the seven cows at different stages are shown in Fig. 36. The value at stage five is significantly lower (P = 0.05) than all other stages except stage six which is significantly lower (P = 0.05) than stages eight and nine.

Beta-hydroxybutyric Acid The range of values recorded was from 0.07 to 0.80 mg./100 ml., as acetone, with a mean

FIG. 37.

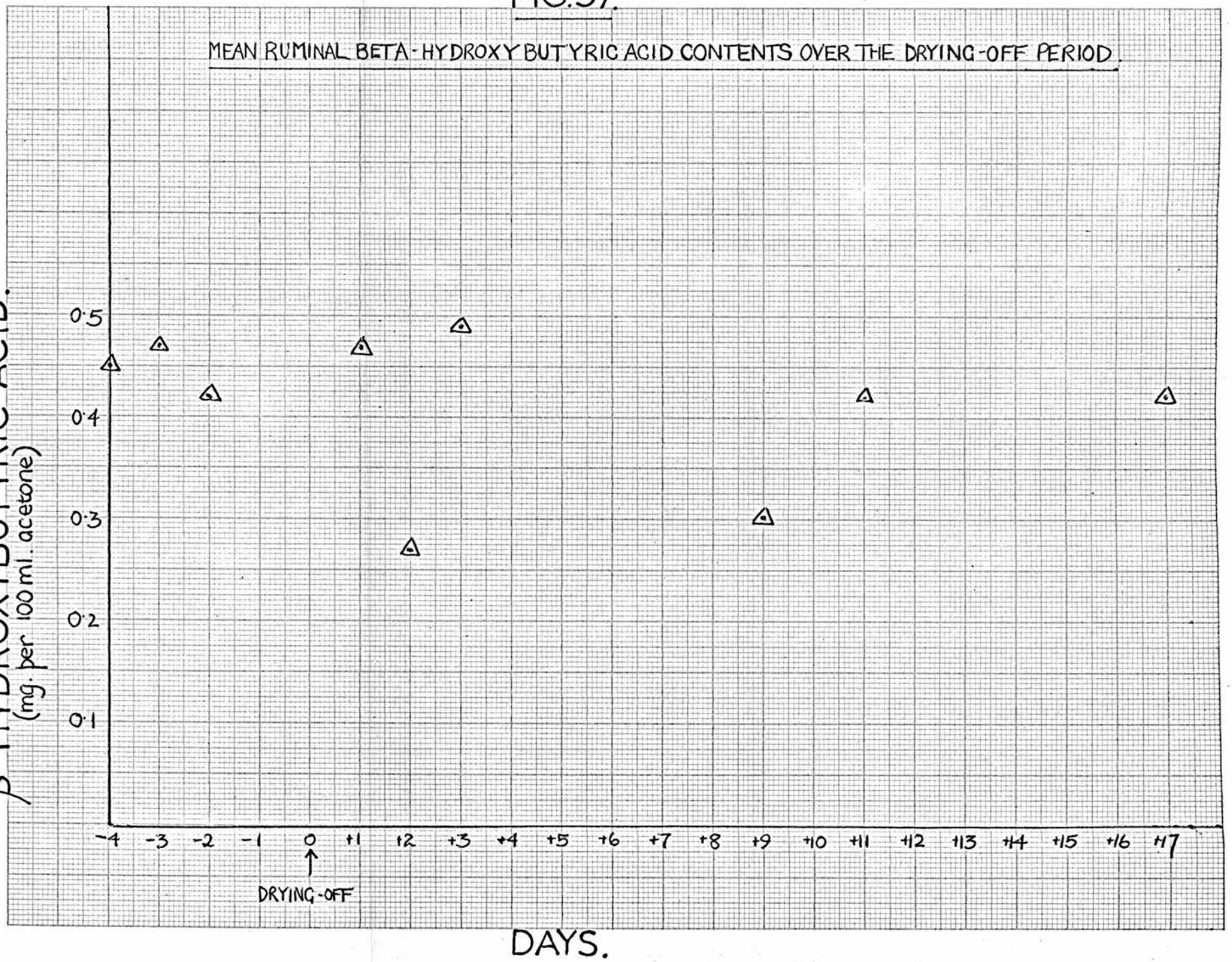

MEAN RUMINAL BETA-HYDROXY BUTYRIC ACID CONTENTS OVER THE DRYING-OFF PERIOD.
B-HYDROXYBUTYRIC ACID. (mg. per 100 ml. acetone)
0·5
0·4
0·3
0·2
0·1
-4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8 +9 +10 +11 +12 +13 +14 +15 +16 +17
DRYING-OFF
DAYS.

FIG 38

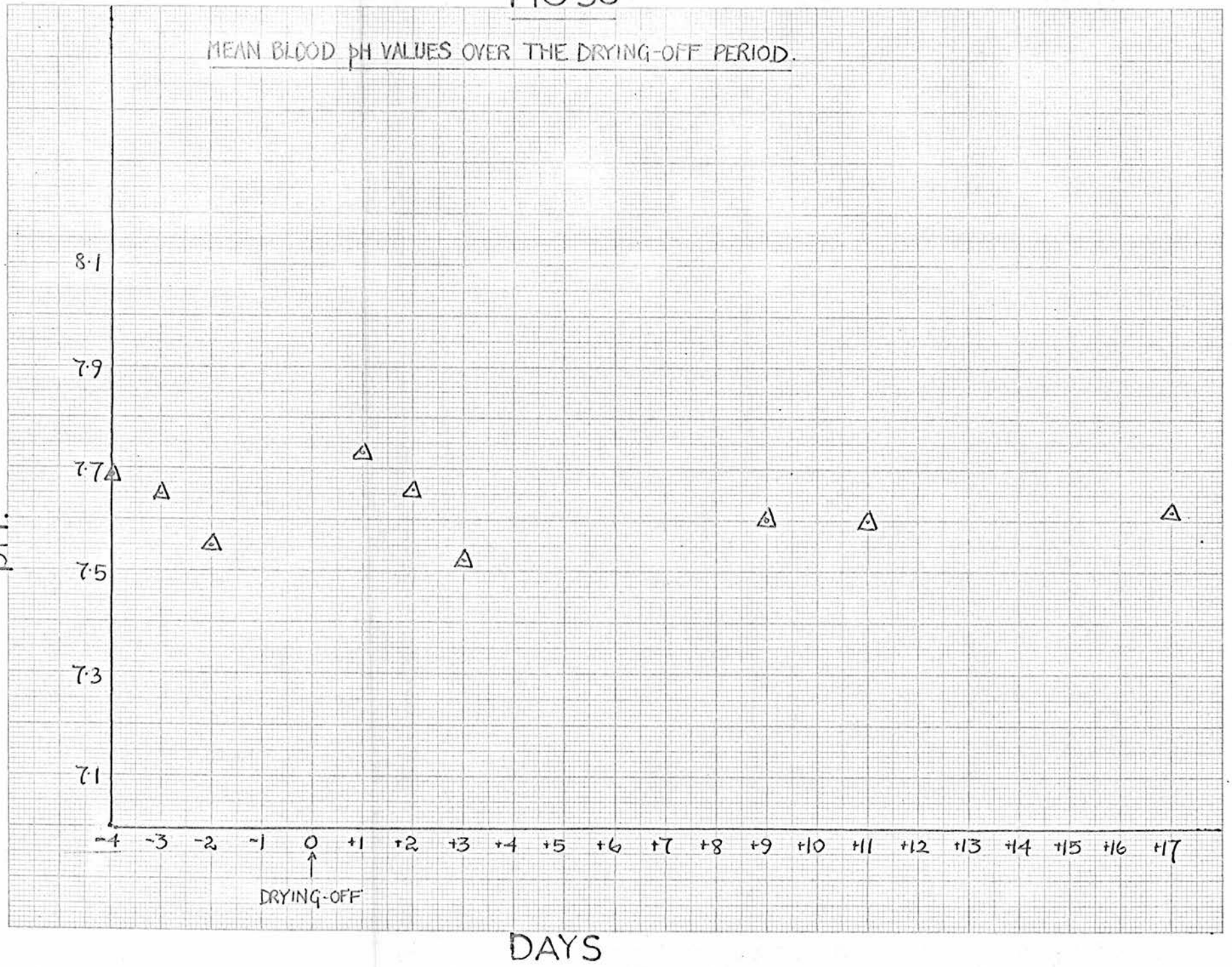
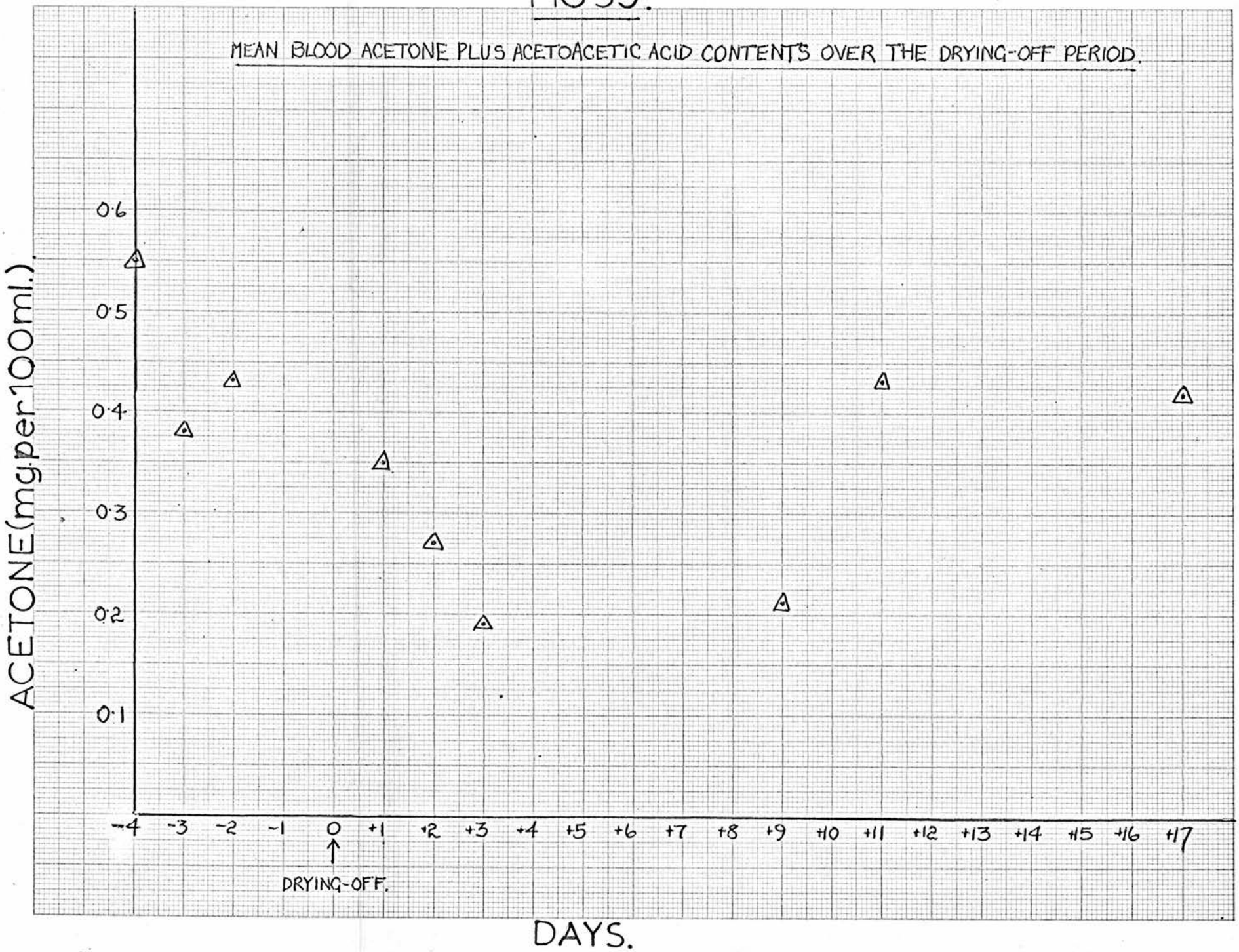

MEAN BLOOD pH VALUES OVER THE DRYING-OFF PERIOD.
pH
8·1
7·9
7·7
7·5
7·3
7·1
-4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8 +9 +10 +11 +12 +13 +14 +15 +16 +17
DRYING-OFF
DAYS

FIG 39.


MEAN BLOOD ACETONE PLUS ACETOACETIC ACID CONTENTS OVER THE DRYING-OFF PERIOD.
ACETONE (mg. per 100 ml.).
0·6
0·5
0·4
0·3
0·2
0·1
-4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8 +9 +10 +11 +12 +13 +14 +15 +16 +17
DRYING-OFF.
DAYS.

value of 0.41 mg./100 ml. An analysis of variance gave significant evidence for differences between cows (P = 0.001) and between stages (P = 0.05). Mean values for the seven cows at different stages are shown in Fig. 37. The value at stage five is significantly lower (P = 0.05) than all others except that at stage seven which is significantly lower (P = 0.05) than those at stages one, two, four and six.

Blood

The results of the analyses carried out on the blood samples are given in Appendix Tables 51, 54, 57, 60, 63, 66 and 69.

pH The range of pH values was from 7.03 to 7.90 with a mean of 7.63. An analysis of variance gave significant evidence of differences between cows but not between stages. The mean pH values for the seven cows at different stages are given in Fig. 38.

Acetone + Acetoacetic Acid The range of values was from 0.01 to 1.29 mg./100 ml., as acetone, with a mean value of 0.36 mg./100 ml. An analysis of variance gave significant evidence (P = 0.001) for differences between cows and between stages. The mean values for the seven cows at different stages are shown in Fig. 39. Values at stages six and seven are significantly lower (P = 0.05) than at all others except stage five which is significantly lower (P = 0.05) than all others except those at two and four.

Beta-hydroxybutyric Acid The values for individual animals cover a range from 0.35 to 3.85 mg./100 ml., as acetone, with a mean of 1.75 mg./100 ml. An analysis of variance gave significant evidence for differences between cows (P = 0.001)

FIG. 40.

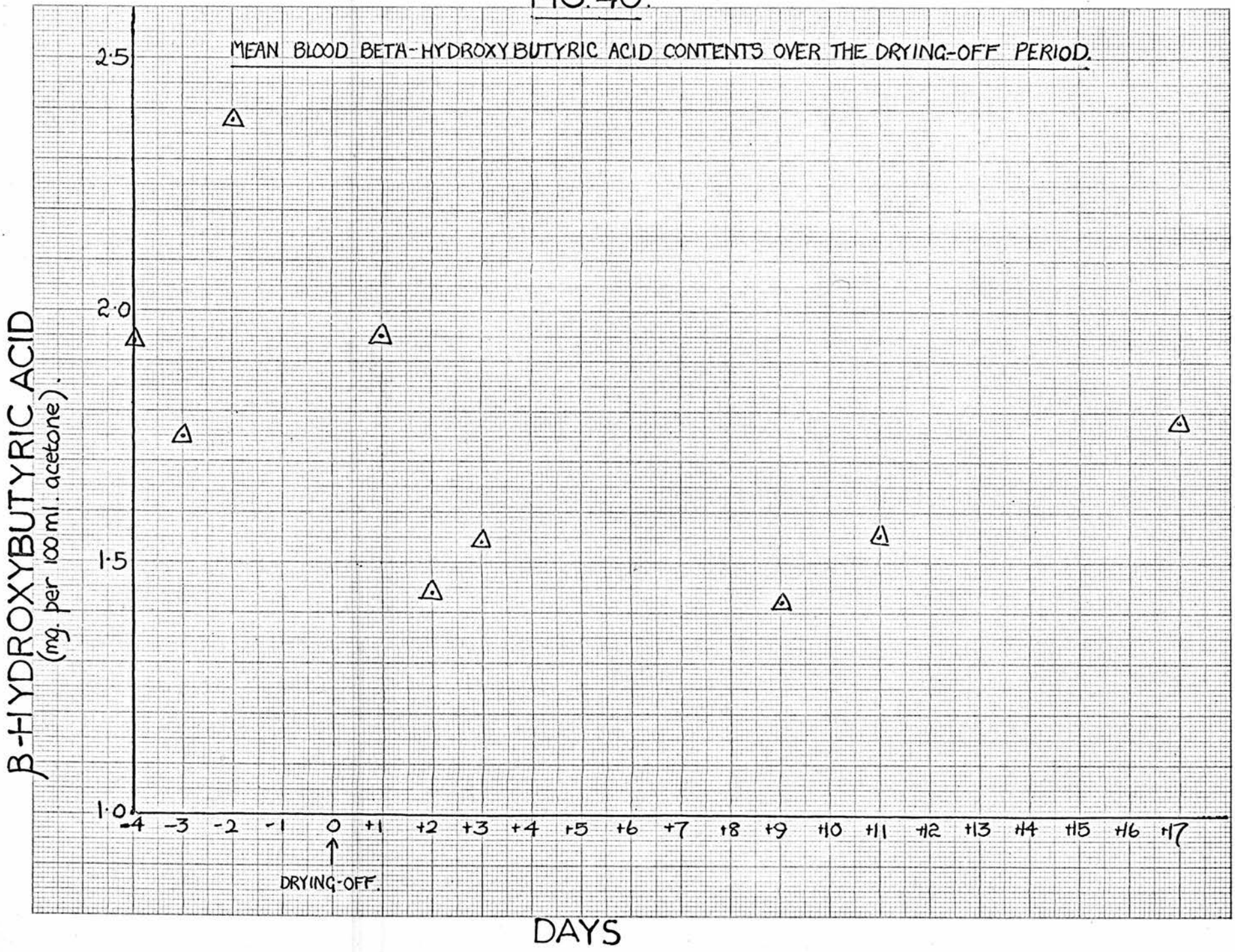

MEAN BLOOD BETA-HYDROXY BUTYRIC ACID CONTENTS OVER THE DRYING-OFF PERIOD.
β-HYDROXYBUTYRIC ACID
(mg. per 100 ml. acetone).
2·5
2·0
1·5
1·0
-4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8 +9 +10 +11 +12 +13 +14 +15 +16 +17
DRYING-OFF.
DAYS

FIG.41.

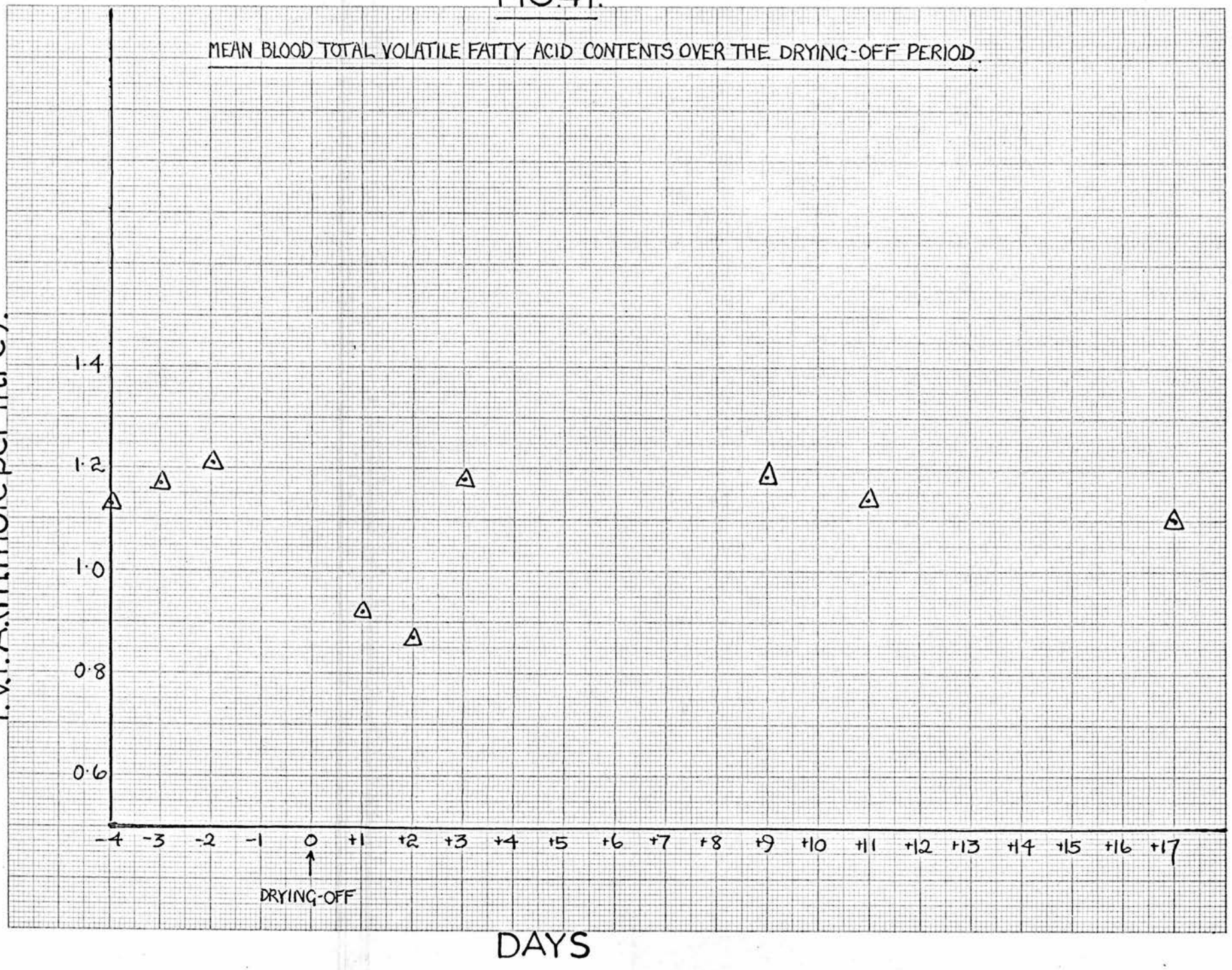
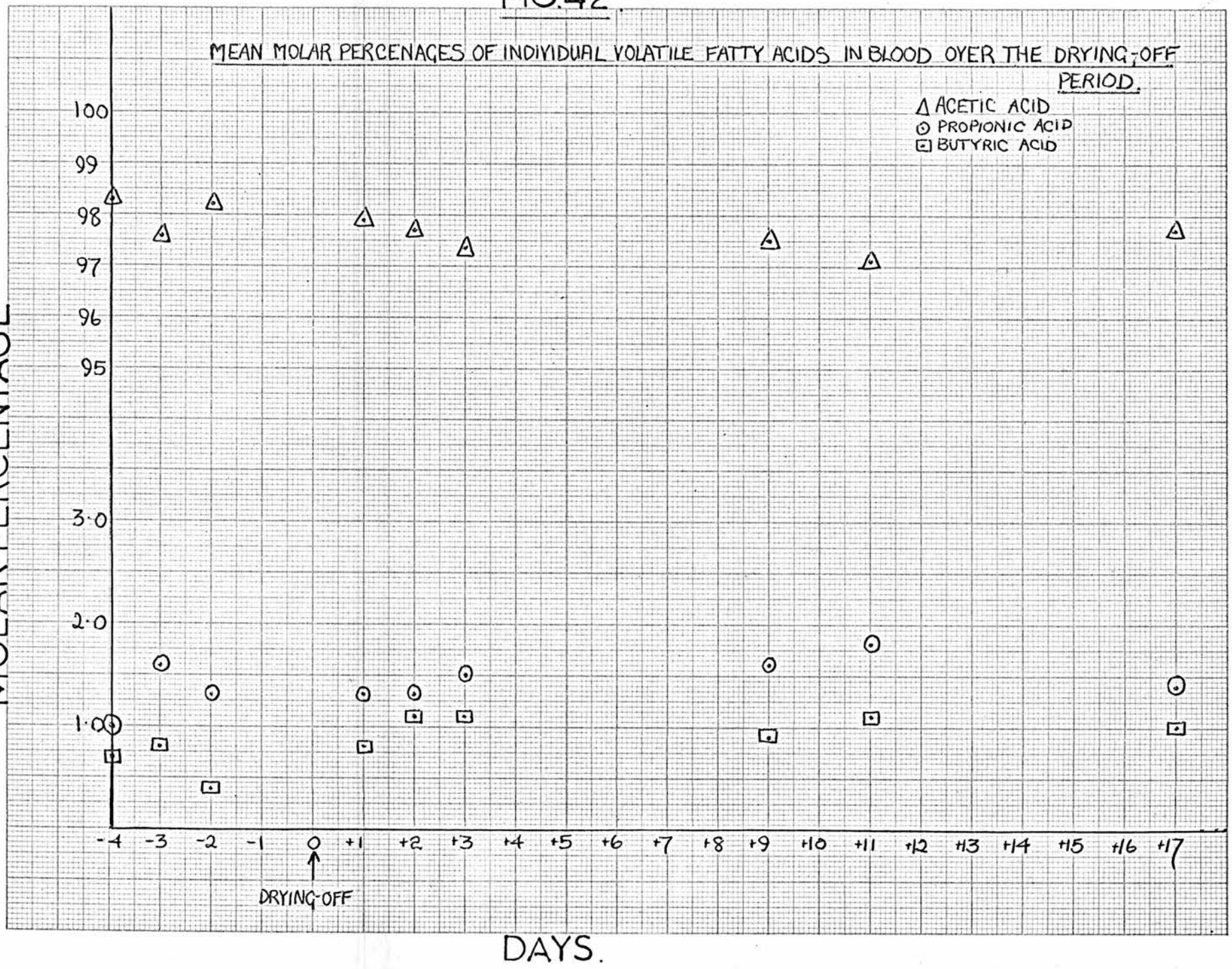

MEAN BLOOD TOTAL VOLATILE FATTY ACID CONTENTS OVER THE DRYING-OFF PERIOD.
T.V.F.A.(m.mole per litre).
1·4
1·2
1·0
0·8
0·6
-4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8 +9 +10 +11 +12 +13 +14 +15 +16 +17
DRYING-OFF
DAYS

FIG. 42.


MEAN MOLAR PERCENAGES OF INDIVIDUAL VOLATILE FATTY ACIDS IN BLOOD OVER THE DRYING-OFF PERIOD.
ACETIC ACID
PROPIONIC ACID
BUTYRIC ACID
MOLAR PERCENTAGE
100
99
98
97
96
95
3·0
2·0
1·0
-4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8 +9 +10 +11 +12 +13 +14 +15 +16 +17
DRYING-OFF
DAYS.

and between stages (P = 0.01). The mean values for the seven cows at different stages are given in Fig. 40. There is a low level of beta-hydroxybutyric acid at the four stages following drying-off. Values at stages five and seven are significantly lower (P = 0.05) than those at stages one, three and four. Values at stages six and eight are significantly lower (P = 0.05) than that at stage three.

Total Volatile Fatty Acids The range of values was from 0.70 to 1.66 m.mole/litre with a mean of 1.10 m.mole/litre. An analysis of variance gave significant evidence for differences between stages (P = 0.05) but not between cows. Mean values for the seven cows at different stages are shown in Fig. 41. The period following drying off shows low values and those at stages four and five are significantly lower (P = 0.05) than those at all other stages except nine.

Individual Volatile Fatty Acids Analyses of variance gave no significant evidence for differences between cows or between stages in the molar percentages of the volatile fatty acids. The mean values for the seven cows at different stages are given in Fig. 42.

DISCUSSION

The results recorded in this investigation show statistically significant changes in the rumen contents in the period following upon drying-off. Thus total volatile fatty acid content, acetone + acetoacetic acid content and, to a less marked extent, beta-hydroxybutyric acid content are all low at this time.

These changes are not easily explained as it is difficult to see how drying-off per. se. could affect the rumen contents. There was no change in the intake of food or its nature and the rumen changes cannot be explained by dietary variations.

Drying-off results in removal of the demands for raw materials, for milk synthesis, by the mammary gland, although this demand does not cease completely for several days. It would be expected that consequent upon drying-off there would be an increase in the circulating levels of materials like acetic acid and beta-hydroxybutyric acid which are used by the gland. Knodt et al[120] were unable to show any increases in blood ketones following upon drying off and suggested that there was an increased utilisation of beta-hydroxybutyric acid by parts of the body other than the mammary gland. The present data, show levels of acetic acid and ketones, after drying-off, which are significantly lower than normal. A depletion of circulating levels of these metabolites resulting from increased demands for them by the body after drying-off does not seem a reasonable explanation since the animals were being sufficiently well fed to deal with any nutrient demands made by them at this time. It appears that there is a period of adjustment required after drying-off during which the blood changes recorded take place. The physiological and biochemical explanations are not obvious.

The data in Experiment 3 show a closer relationship between ruminal pH and total volatile fatty acid content than in Experiments 1 and 2. The relationship, however, is not close.

The blood pH values reported show a number at 7.90. This is very high indeed and would be regarded by many authorities as the upper limit at which life could exist. None of the animals in the experiment showed any signs of illness at any time.

The volatile fatty acid pattern of the blood confirms the data from the first two experiments in that acetic is dominant and the amounts of butyric and propionic acids are small. On several occasions during the experiment traces of branched chain volatile fatty acids were detected in the blood.

SUMMARY

1. Three experiments were carried out to determine the effect of lactation upon the composition of milk, blood and rumen liquor.
2. Experiment 1 involved six cows from two months before calving to drying-off. Milk, blood and rumen liquor samples were taken at seven days post-partum and at two-weekly intervals thereafter. Milk samples were analysed for fat, total solids, lactose, crude protein and chloride content. Blood and rumen liquor samples were analysed for pH, acetone plus acetoacetic acid, beta-hydroxybutyric acid, total volatile fatty acids and individual volatile fatty acid content. Conditions were so controlled that variations in milk and blood composition could justifiably be regarded as true lactation effects occasioned by the physiological changes associated with lactation.
3. Rumen liquors showed considerable differences in composition but no statistically significant differences between animals or stages of lactation were demonstrable. No lactation trends were discernible. There were no correlations between milk and rumen constituents.
4. Milk yield and lactose content rose to a maximum in early lactation and fell throughout the remainder of the lactation. Fat, total solids and crude protein contents fell in early lactation then rose to a level which remained relatively constant before rising sharply in late lactation. Changes in late lactation coincided with the twentieth week of pregnancy.
5. Blood levels of beta-hydroxybutyric acid and acetic acid rose

to maxima in early lactation, declined sharply and then remained relatively constant. These changes were not related to changes in rumen liquors. High levels coincided with the period in lactation when production was at a maximum and it is suggested that they result from a shortage of glucose. This would have the effect of reducing utilisation of acetyl coenzyme A via the tricarboxylic acid cycle and result in production of ketone bodies. At the same time lipogenesis would be reduced and acetic acid and beta-hydroxybutyric acid would tend to accumulate as a result. Reduction of mammary gland lipogenesis would account, in part, for the low fat content of milk at this stage of lactation.

6. Experiment 2 involved nine cows from two months before calving until sixteen days post-partum. Samples of rumen liquor and blood were taken on three consecutive days in the week before calving, on the day of calving, on the two following days and on the eighth, tenth and sixteenth days post-partum. Samples were analysed as in Experiment 1.

7. Lowered food intakes at parturition and immediately following resulted in low ruminal total volatile fatty acid contents and there was some evidence for higher acetic acid and lower propionic acid contents in these samples.

8. The blood samples showed temporarily high beta-hydroxybutyric acid and acetic acid contents at parturition. This may have resulted from glucose mobilisation caused by stress with consequent depletion of liver glycogen and increased fat

catabolism. The rises in acetic acid and beta-hydroxybutyric acid levels from low post-partum levels are typical of the lactation trends demonstrated in Experiment 1.

9. In Experiment 3 seven cows were used from four weeks before drying-off to seventeen days after drying-off. Samples of blood and rumen liquor were taken on three consecutive days in the week before drying-off and on the first, second, third, ninth, eleventh and seventeenth days after drying off. Samples were analysed as in Experiments 1 and 2.

10. Rumen liquor samples showed significant changes in the post-drying-off period. These could not be accounted for by dietary changes.

11. There were significant changes in the levels of acetic acid, acetone + acetoacetic acid and beta-hydroxybutyric acid in the blood, all showing low values in the post-drying-off period. It is suggested that these changes are the result of the animal adjusting itself to the changed demands resulting from the cessation of the synthetic activities of the mammary gland, and achieving a new physiological balance.

APPENDIX I

TABLE 1.

Yield and Composition of Milk for Cow No. 1.

Week	Yield lb.	Fat %	Solids-not-fat %	Crude Protein %	Lactose %	Chloride mg/100ml
1	41.5	4.39	9.74	4.44	4.55	64
3	42.0	4.15	9.26	3.80	4.75	76
5	43.0	3.70	8.89	3.37	4.80	83
7	38.0	4.22	9.07	3.64	4.77	81
9	32.5	4.45	9.12	3.56	4.81	76
11	27.5	4.54	8.97	3.45	4.90	70
13	26.0	4.60	9.25	3.75	4.80	86
15	29.5	4.55	9.24	3.67	4.82	81
17	27.5	4.30	9.23	3.84	4.65	83
19	25.5	4.31	9.20	3.76	4.70	83
21	26.0	4.31	9.05	3.75	4.75	78
23	22.0	4.28	8.88	3.58	4.59	82
25	22.5	4.37	8.95	3.78	4.52	86
27	22.0	4.36	8.92	3.75	4.45	97
29	21.0	4.40	8.81	3.85	4.30	104
31	17.5	4.46	9.15	4.22	4.19	111
33	12.0	4.70	9.34	4.56	4.15	115
35	10.0	4.75	9.23	4.52	4.00	126

TABLE 2

Composition of Blood Samples from Cow No. 1.

Week	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.38	5.76	2.91	0.75	95.5	2.5	2.0
3	7.60	4.50	5.11	0.91	94.4	2.8	2.8
5	7.42	2.25	7.84	1.28	94.0	2.4	3.6
7	7.42	4.95	8.48	1.23	94.5	2.8	2.7
9	7.40	5.04	7.60	1.10	93.0	3.1	3.9
11	7.45	9.90	6.90	1.05	87.5	5.3	7.2
13	7.50	4.50	4.65	0.76	94.5	2.0	3.5
15	7.48	4.23	3.46	0.80	89.2	4.4	6.4
17	7.55	0.45	4.25	0.66	94.5	3.3	2.2
19	7.68	2.70	2.85	0.82	93.2	2.4	4.4
21	7.43	0.45	2.90	0.80	95.5	1.9	2.6
23	7.45	5.76	3.90	0.65	97.0	2.2	0.8
25	7.44	3.24	3.80	0.87	97.3	1.6	1.1
27	7.45	0.00	1.55	0.77	94.0	2.2	3.8
29	7.55	4.68	2.49	0.74	95.2	3.0	1.8
31	7.40	3.35	3.78	0.87	96.0	1.0	3.0
33	7.45	2.95	2.95	0.75	97.0	1.5	1.5
35	7.50	1.44	3.55	0.70	90.6	1.2	8.2

TABLE 3

Composition of Rumen Liquor Samples from Cow No. 1.

Week	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.70	2.70	0.56	66.8	41.6	12.4	1.0	7.2	1.7	0.9
3	6.45	3.15	0.33	68.8	42.7	10.2	0.5	11.3	2.3	1.1
5	6.78	1.17	0.83	71.2	44.8	12.0	0.9	12.0	1.5	1.2
7	6.82	0.00	0.09	52.5	31.8	9.7	0.6	8.0	1.0	0.6
9	6.78	0.00	0.06	57.7	38.4	9.7	1.0	7.6	1.6	0.7
11	6.90	0.00	0.35	73.5	44.7	12.0	1.7	9.6	2.3	1.1
13	6.35	2.25	1.02	95.0	52.1	16.0	1.4	16.4	2.9	2.2
15	6.10	7.02	0.30	88.1	51.5	12.3	1.4	15.0	2.5	1.8
17	6.60	5.04	1.02	87.2	51.8	12.3	1.5	15.2	2.5	1.4
19	6.72	0.27	0.38	80.0	50.0	12.6	1.6	10.1	2.4	1.7
21	6.95	3.42	0.34	75.0	43.4	12.4	1.7	9.8	2.0	1.4
23	6.45	5.49	0.46	76.0	44.8	13.0	1.4	11.5	2.8	1.4
25	6.55	0.54	0.18	84.1	49.7	13.7	1.2	11.2	2.3	1.0
27	6.50	4.50	0.90	85.9	53.9	14.2	1.8	11.0	1.8	1.0
29	6.56	0.00	0.02	79.0	50.6	12.7	2.0	8.9	2.5	0.8
31	6.23	0.56	0.90	83.7	53.6	9.2	2.3	10.0	3.3	1.0
33	6.40	1.45	0.40	81.0	51.0	12.2	1.9	11.8	3.3	1.3
35	6.50	6.37	0.37	56.5	33.9	7.9	1.5	8.1	1.8	1.0

TABLE 4.

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 1.

Week	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	64.2	19.2	1.5	11.1	2.7	1.3
3	62.3	14.9	0.8	16.5	3.4	2.1
5	61.9	16.5	1.2	16.6	2.0	1.8
7	61.5	18.8	1.1	15.4	2.0	1.2
9	65.1	16.4	1.7	12.9	2.7	1.2
11	62.6	16.9	2.3	13.5	3.2	1.5
13	57.2	17.6	1.5	18.1	3.2	2.4
15	60.2	14.4	1.6	17.5	2.9	2.0
17	61.3	14.5	1.7	17.9	3.0	1.6
19	63.9	16.1	2.0	12.9	3.0	2.1
21	61.4	17.5	2.4	13.8	2.8	2.1
23	59.9	17.4	1.8	15.3	3.7	1.9
25	62.8	17.3	1.5	14.2	2.9	1.2
27	64.4	16.9	2.1	13.2	2.1	1.3
29	65.3	16.4	2.5	11.5	3.2	1.1
31	66.7	13.0	2.4	13.2	3.1	1.6
33	62.6	14.5	2.7	15.0	3.4	1.8
35	65.6	13.8	2.3	14.1	2.8	1.4

TABLE 5

Yield and Composition of Milk for Cow No. 2

Week	Yield lb.	Fat %	Solids-not-fat %	Crude Protein %	Lactose %	Chloride mg/100ml
1	41.0	4.92	9.48	4.31	4.43	79
3	56.5	4.80	8.60	3.33	4.70	71
5	55.0	4.15	8.30	2.82	4.90	56
7	46.5	3.95	8.24	2.67	5.00	67
9	43.0	3.70	8.40	3.02	4.87	81
11	41.0	4.50	8.46	2.96	4.87	81
13	40.0	4.60	8.48	3.05	4.70	71
15	36.5	4.55	8.70	3.28	4.75	82
17	33.5	4.72	8.66	3.34	4.67	77
19	27.0	4.76	8.65	3.25	4.71	89
21	24.0	4.85	8.57	3.36	4.58	92
23	20.5	4.90	8.60	3.48	4.60	103
25	21.0	5.05	8.60	3.50	4.55	106
27	19.0	5.10	8.75	3.63	4.50	120
29	12.0	5.09	8.90	3.88	4.39	126
31	10.0	5.10	9.00	3.99	4.43	152
33	8.0	5.15	9.05	4.15	4.30	150
35	7.5	5.35	9.08	4.28	4.17	155

TABLE 6

Composition of Blood Samples from Cow No. 2.

Week	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.48	2.42	4.25	0.87	94.2	2.1	3.7
3	7.48	1.80	13.90	0.80	92.6	3.0	4.4
5	7.53	3.24	27.37	1.62	93.5	2.7	3.8
7	7.55	3.87	8.00	1.30	95.5	2.2	2.3
9	7.56	5.76	5.56	1.14	92.6	3.1	4.3
11	7.50	2.34	3.96	0.58	89.2	5.1	5.7
13	7.60	3.51	3.67	0.85	93.6	2.2	4.2
15	7.73	0.99	4.87	0.75	88.5	4.8	6.7
17	7.44	4.05	4.35	0.62	91.2	4.0	4.8
19	7.49	2.25	5.29	0.80	94.1	2.3	3.6
21	7.40	0.72	3.98	0.85	95.5	2.5	2.0
23	7.60	4.05	3.14	0.72	97.0	2.2	0.8
25	7.63	0.45	3.84	0.70	96.8	1.7	1.5
27	7.41	1.17	4.31	0.59	91.5	2.6	5.9
29	7.45	2.35	3.56	0.76	94.7	3.2	2.1
31	7.50	0.42	3.18	0.68	95.8	1.8	2.4
33	7.48	2.20	4.20	0.56	96.0	1.8	2.2
35	7.45	2.52	4.05	0.60	91.5	1.1	7.4

TABLE 7.

Composition of Rumen Liquor Samples from Cow No. 2

Week	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.38	4.20	0.35	82.7	51.7	10.9	1.5	15.3	1.7	1.5
3	6.65	3.87	0.40	57.3	36.8	6.7	0.4	9.9	0.3	0.5
5	6.80	2.70	1.43	68.4	45.9	10.2	1.5	8.8	1.2	0.7
7	6.82	3.42	0.00	67.5	45.1	8.0	1.3	10.3	1.8	0.7
9	6.41	1.98	0.16	75.9	43.2	11.5	1.1	14.5	1.4	1.9
11	6.60	1.17	0.24	76.5	43.3	11.4	1.0	12.4	1.5	0.9
13	6.72	0.00	0.11	66.8	41.9	9.1	1.0	12.4	1.5	0.9
15	6.55	5.85	1.28	85.2	47.9	12.8	1.4	15.4	1.9	1.7
17	6.50	7.02	1.10	68.9	44.0	9.8	0.9	9.8	1.0	0.8
19	6.60	0.34	0.08	76.5	50.4	11.4	1.1	11.6	1.2	0.9
21	6.40	2.79	0.30	86.7	56.5	13.0	1.3	13.3	1.3	1.2
23	6.52	3.60	1.13	66.0	44.2	9.7	1.2	11.0	2.1	1.0
25	6.68	4.95	1.55	78.9	50.9	10.0	0.8	11.0	1.4	0.8
27	6.55	2.25	0.41	65.8	40.3	8.4	1.5	11.1	2.3	1.0
29	6.60	1.25	0.12	67.5	42.0	9.2	1.3	12.0	2.1	0.9
31	6.55	0.18	0.90	61.9	40.3	8.4	1.3	10.6	1.0	0.9
33	6.70	0.78	1.10	65.9	41.0	9.5	1.4	10.9	2.0	1.1
35	6.75	3.67	0.30	70.5	45.5	9.6	1.0	11.9	1.1	1.1

TABLE 8.

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 2.

Week	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	62.6	13.2	1.8	18.6	2.0	1.8
3	67.4	12.2	0.7	18.1	0.6	1.0
5	67.3	14.9	2.2	12.9	1.8	0.9
7	67.2	11.9	1.9	15.3	2.6	1.1
9	58.7	15.7	1.5	19.7	1.9	2.5
11	59.4	15.6	1.4	19.2	1.9	2.5
13	62.7	13.7	1.5	18.5	2.3	1.3
15	59.1	15.8	1.7	19.0	2.3	2.1
17	66.4	14.8	1.4	14.8	1.6	1.0
19	65.7	14.9	1.5	15.1	1.6	1.2
21	65.4	15.0	1.5	15.3	1.5	1.3
23	64.0	14.0	1.7	15.9	3.1	1.3
25	67.9	13.3	1.1	14.7	1.9	1.1
27	62.4	13.0	2.3	17.2	3.5	1.6
29	63.4	13.3	2.1	17.1	2.5	1.6
31	64.5	13.6	2.0	16.9	1.5	1.5
33	65.6	13.5	1.8	16.5	1.4	1.2
35	64.8	13.0	2.2	16.0	1.8	1.2

TABLE 9.

Yield and Composition of Milk for Cow No. 3.

Week	Yield lb.	Fat %	Solids-not-fat %	Crude Protein %	Lactose %	Chloride mg/100ml
1	40.0	4.36	9.36	4.02	4.70	78
3	42.5	3.98	9.17	3.45	5.00	69
5	42.0	3.95	9.05	3.26	5.11	71
7	40.5	4.00	8.66	2.93	5.03	64
9	37.0	3.99	8.57	2.88	5.05	65
11	36.0	4.10	8.98	3.29	5.00	65
13	33.0	4.07	8.90	3.35	4.86	70
15	30.0	4.00	8.87	3.46	4.80	74
17	28.5	4.10	8.87	3.37	4.85	72
19	26.5	3.95	8.90	3.33	4.90	73
21	29.0	4.03	8.76	3.32	4.81	73
23	29.5	3.99	8.80	3.28	4.85	73
25	28.0	3.79	8.82	3.43	4.73	78
27	27.0	3.90	8.95	3.60	4.65	78
29	25.5	3.90	9.05	3.61	4.72	78
31	25.0	4.20	8.93	3.77	4.43	80
33	24.5	4.30	9.15	4.14	4.38	80
35	22.0	4.29	9.45	4.64	4.18	76
37	20.0	4.25	8.92	3.77	4.40	101
39	14.5	4.60	9.04	3.81	4.43	90
41	17.0	3.95	9.05	3.73	4.57	108
43	14.0	4.79	8.94	3.84	4.35	103
45	14.0	4.59	9.08	3.90	4.38	97
47	13.0	4.30	9.21	4.11	4.30	108

TABLE 10

Composition of Blood Samples from Cow No. 3

Week	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.40	3.78	4.29	0.78	96.0	2.4	1.6
3	7.55	1.45	4.62	0.63	96.0	2.5	1.5
5	7.45	2.34	11.92	1.15	96.0	1.9	2.1
7	7.45	0.63	5.60	1.45	96.7	2.1	1.2
9	7.58	5.40	0.80	1.38	95.7	2.7	1.6
11	7.50	11.25	4.25	0.95	88.5	5.1	6.4
13	7.58	0.19	3.78	0.85	95.2	1.9	2.9
15	7.40	0.63	3.81	0.75	90.2	4.2	5.6
17	7.62	0.45	5.08	0.92	92.6	3.4	4.0
19	7.60	3.78	2.48	1.04	92.6	2.6	4.8
21	7.43	1.44	5.85	0.92	91.7	3.0	5.3
23	7.47	1.89	4.32	0.84	93.5	3.1	3.4
25	7.39	0.90	3.60	1.04	95.7	1.9	2.4
27	7.62	0.45	3.55	1.04	92.2	2.5	5.3
29	7.58	1.17	3.78	0.92	93.2	3.4	3.4
31	7.44	1.62	2.94	1.05	94.9	0.9	4.2
33	7.39	2.25	4.23	0.61	93.4	1.9	4.7
35	7.50	9.45	5.39	0.73	92.9	1.5	5.6
37	7.46	0.34	2.63	0.92	93.0	4.8	2.2
39	7.52	0.38	4.19	0.70	91.5	6.5	2.0
41	7.50	0.00	4.25	0.65	92.7	5.8	1.5
43	7.45	0.45	3.67	0.85	94.0	4.6	1.4
45	7.55	0.40	2.95	0.95	90.5	7.0	2.5
47	7.60	0.29	3.10	1.02	91.0	4.0	5.0

TABLE 11.

Composition of Rumen Liquor Samples from Cow No. 3.

Week	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.55	0.00	0.89	77.5	49.7	14.1	1.1	8.7	1.3	1.0
3	6.85	2.25	1.08	60.6	36.5	8.5	1.4	9.6	1.9	1.7
5	6.70	4.95	0.88	72.6	44.9	8.1	1.2	14.5	1.7	1.3
7	6.78	2.25	1.05	66.7	41.7	9.9	0.9	10.0	1.0	0.7
9	6.60	0.90	0.08	60.0	38.4	8.7	1.4	8.7	1.2	0.6
11	6.70	3.42	0.10	88.2	61.4	13.7	1.5	5.7	2.2	1.8
13	6.66	4.68	0.42	83.7	49.4	12.1	1.1	15.7	2.0	1.3
15	6.65	0.50	0.39	65.4	41.2	10.2	1.1	8.0	1.9	1.4
17	6.60	0.90	0.05	90.1	52.2	14.9	1.4	15.6	2.2	2.0
19	6.70	4.95	0.80	70.3	43.5	9.8	1.4	10.4	1.1	1.9
21	6.55	6.57	0.17	69.1	46.4	9.2	1.0	10.6	1.0	0.6
23	6.70	0.63	0.00	64.0	40.8	9.8	1.2	10.0	1.6	0.8
25	6.61	3.78	1.29	66.6	43.7	9.5	1.0	9.4	1.4	0.8
27	6.63	3.06	0.14	75.5	48.0	13.6	0.8	10.8	1.3	0.9
29	6.65	3.60	0.17	81.1	49.0	14.7	1.1	11.1	2.1	1.3
31	6.53	4.14	0.90	84.6	48.1	12.7	1.1	12.5	2.0	1.4
33	6.42	3.15	1.59	80.5	51.3	11.7	1.5	11.7	1.6	0.9
35	6.58	1.08	1.29	73.4	44.3	10.1	1.9	12.1	2.5	1.3
37	6.75	0.00	0.05	75.0	45.9	11.2	1.1	14.8	1.5	0.5
39	6.40	0.05	1.20	82.0	51.6	15.0	1.3	12.5	1.7	0.9
41	6.85	1.56	0.32	78.0	48.9	10.5	1.5	14.3	0.9	1.9
43	6.60	2.35	0.57	68.0	45.2	9.5	1.2	7.9	2.2	2.0
45	6.55	3.20	0.28	72.0	42.5	13.5	1.0	12.5	1.8	0.7
47	6.80	2.67	0.75	70.0	43.0	12.5	1.1	11.3	1.3	0.8

TABLE 12.

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 3.

Week	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	65.8	18.5	1.7	11.5	1.7	1.0
3	61.2	14.3	1.6	16.2	3.1	2.9
5	62.6	11.3	1.9	20.2	2.4	1.8
7	64.9	15.4	2.7	15.6	1.6	1.1
9	65.2	14.8	1.8	14.8	2.0	0.8
11	61.2	15.9	2.5	16.6	2.5	2.1
13	60.6	14.8	1.2	19.3	2.4	1.6
15	64.7	15.9	2.3	12.5	2.9	2.3
17	59.1	16.9	1.4	17.6	2.5	2.3
19	63.1	14.2	1.6	15.0	2.9	2.7
21	67.4	13.3	1.9	15.5	1.5	0.9
23	63.5	15.2	1.5	15.6	2.6	1.2
25	66.3	14.4	1.6	14.3	2.1	1.4
27	63.7	18.1	1.2	14.3	1.7	1.1
29	61.9	18.6	1.0	14.0	2.6	1.5
31	61.8	16.4	1.5	16.1	2.6	1.7
33	65.2	14.9	2.1	14.8	2.1	1.1
35	61.3	14.0	2.6	16.8	3.4	1.8
37	61.2	14.9	1.5	19.7	2.0	0.7
39	62.2	18.1	1.6	15.1	2.1	1.1
41	62.7	13.5	1.9	18.3	1.2	2.4
43	66.5	14.0	1.8	11.6	3.2	2.9
45	59.0	18.8	1.4	17.4	2.5	1.0
47	61.4	17.9	1.6	16.1	1.9	1.1

TABLE 13

Yield and Composition of Milk for Cow No. 4

Week	Yield lb.	Fat %	Solids-not-fat %	Crude Protein %	Lactose %	Chloride mg/100ml
1	45.0	4.58	9.23	3.87	4.58	85
3	48.5	4.06	8.76	3.17	5.04	85
5	42.5	3.45	8.54	3.05	4.94	80
7	42.5	4.14	8.46	2.86	4.90	75
9	38.0	4.30	8.45	3.00	4.75	70
11	29.0	4.32	8.57	3.19	4.68	80
13	25.0	4.31	8.59	3.36	4.56	72
15	23.0	4.13	8.69	3.50	4.49	78
17	24.5	4.12	8.64	3.49	4.46	70
19	23.0	4.06	8.66	3.76	4.37	82
21	24.5	4.06	8.68	3.65	4.34	80
23	23.0	3.95	8.62	3.64	4.29	70
25	21.0	3.92	8.77	3.73	4.26	65
27	23.0	3.90	8.81	3.84	4.24	68
29	18.5	3.81	8.83	3.88	4.27	75
31	19.0	4.20	9.06	4.16	4.16	85
33	16.0	4.49	9.13	4.30	4.15	80
35	14.0	4.42	9.19	4.43	4.06	92
37	10.0	4.45	9.25	4.40	4.05	105

TABLE 14.

Composition of Blood Samples from Cow No. 4

Week	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.60	9.90	2.57	0.61	95.0	2.7	2.3
3	7.64	4.95	3.91	0.72	95.0	2.7	2.3
5	7.42	4.23	4.91	0.82	92.5	2.9	4.6
7	7.63	2.97	10.19	0.81	93.2	3.0	3.8
9	7.55	0.36	5.98	1.04	94.5	2.8	2.7
11	7.48	0.18	3.39	1.14	91.5	4.7	3.8
13	7.70	0.90	3.70	0.70	95.0	2.0	3.0
15	7.58	2.52	3.71	0.45	91.5	3.7	4.8
17	7.60	0.90	2.35	0.28	91.4	3.8	4.8
19	7.52	1.08	2.82	0.38	91.3	2.7	6.0
21	7.68	0.00	2.35	0.45	92.6	2.3	5.1
23	7.72	2.70	3.71	0.66	94.7	2.4	2.9
25	7.49	1.53	2.77	0.70	95.2	2.2	2.6
27	7.48	1.17	4.45	0.60	93.6	2.3	4.1
29	7.41	0.00	3.28	0.70	93.0	3.7	3.3
31	7.64	0.00	3.64	0.67	93.5	1.3	5.2
33	7.38	0.18	2.90	0.51	94.5	1.8	3.7
35	7.42	0.45	2.69	0.38	93.3	1.3	5.4
37	7.50	0.35	2.80	0.42	93.5	1.2	5.3

TABLE 15.

Composition of Rumen Liquor Samples from Cow No. 4.

Week	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.70	0.00	1.07	56.5	35.4	9.1	0.9	7.4	1.0	0.9
3	6.80	6.75	0.42	61.0	37.1	8.9	1.0	11.4	1.2	1.0
5	6.72	3.15	0.05	90.7	53.3	18.3	1.7	11.3	1.9	1.6
7	6.60	0.90	0.13	78.1	47.9	10.3	2.1	14.3	2.2	1.0
9	6.90	3.60	0.50	66.4	40.3	10.3	1.2	13.0	1.2	0.5
11	6.70	3.42	0.32	70.5	43.2	10.5	1.7	11.0	2.2	1.0
13	6.65	11.07	0.05	65.8	43.2	9.2	0.8	8.7	1.3	0.7
15	6.70	1.80	0.22	73.1	45.7	10.7	1.6	10.2	1.7	1.0
17	6.65	4.68	0.32	67.5	41.1	10.2	0.9	12.8	1.2	1.4
19	6.50	0.00	0.33	60.7	37.9	9.6	1.0	9.8	1.1	1.3
21	6.62	0.61	0.39	78.3	46.3	11.1	1.4	15.1	2.2	1.3
23	6.55	2.16	0.49	69.2	43.8	10.0	1.0	9.5	1.5	1.5
25	6.35	0.45	0.00	65.6	43.1	8.7	1.0	9.6	1.0	0.7
27	6.79	4.32	1.16	76.6	51.1	12.0	0.9	11.1	1.0	0.5
29	6.70	0.00	0.04	70.5	43.6	13.3	0.7	9.6	1.2	1.2
31	6.81	1.17	1.05	62.7	40.6	9.6	0.9	9.3	1.3	0.7
33	6.90	3.06	0.22	57.2	35.4	7.0	1.1	8.3	1.1	0.3
35	6.81	4.95	0.30	68.0	42.6	9.6	1.7	10.3	1.5	0.7
37	6.85	3.50	0.25	65.0	43.0	13.2	1.1	7.3	0.9	0.5

TABLE 16

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 4.

Week	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	64.8	16.6	1.7	13.5	1.9	1.5
3	61.4	14.7	1.6	18.8	2.0	1.5
5	60.5	20.8	1.9	12.8	2.2	1.8
7	61.6	13.2	2.7	18.4	2.8	1.3
9	60.7	15.5	1.8	19.5	1.8	0.7
11	62.1	15.1	2.5	15.8	3.1	1.4
13	67.7	14.3	1.2	13.7	2.0	1.1
15	64.4	15.1	2.3	14.4	2.4	1.4
17	60.7	15.1	1.4	18.9	1.8	2.1
19	62.4	15.8	1.6	16.1	1.8	2.3
21	59.8	14.4	1.9	19.5	2.9	1.5
23	65.1	14.9	1.5	14.2	2.2	2.1
25	67.2	13.5	1.6	15.0	1.6	1.1
27	66.8	15.6	1.2	14.5	1.3	0.6
29	62.6	19.1	1.0	13.8	1.8	1.7
31	65.0	15.3	1.5	15.0	2.1	1.1
33	66.6	13.2	2.1	15.5	2.1	0.5
35	64.1	14.4	2.6	15.5	2.3	1.1

TABLE 17.

Yield and Composition of Milk for Cow No. 5.

Week	Yield lb.	Fat %	Solids-not-fat %	Crude Protein %	Lactose %	Chloride mg/100ml
1	49.0	4.50	9.38	4.11	4.51	79
3	51.5	4.49	8.87	3.42	4.70	66
5	50.0	4.20	8.75	3.24	4.85	80
7	46.5	3.85	8.53	2.98	4.92	79
9	47.0	3.85	8.30	2.78	4.95	84
11	41.5	3.98	8.44	2.90	4.94	84
13	41.0	3.93	8.62	3.12	4.90	85
15	40.0	4.02	8.79	3.41	4.87	91
17	36.5	3.95	8.80	3.30	4.81	85
19	34.0	4.11	8.84	3.41	4.75	89
21	33.5	4.11	8.77	3.53	4.60	79
23	30.0	3.95	8.60	3.41	4.53	95
25	30.0	4.00	8.67	3.61	4.44	89
27	29.0	3.96	8.66	3.60	4.45	107
29	28.0	4.11	8.65	3.63	4.40	99
31	25.0	4.17	8.51	3.54	4.35	99
33	21.5	4.14	8.65	3.68	4.39	102
35	21.0	4.33	8.72	3.85	4.26	108
37	19.0	3.90	8.64	3.54	4.35	127

TABLE 18

Composition of Blood Samples from Cow No. 5

Week	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.50	5.22	4.65	0.36	96.8	2.0	1.2
3	7.65	0.63	10.93	1.37	94.0	3.0	3.0
5	7.42	6.21	26.58	1.18	94.0	2.1	3.9
7	7.55	3.42	7.06	0.96	94.1	2.9	3.0
9	7.62	3.42	7.02	0.75	91.7	3.3	5.0
11	7.60	9.00	7.61	0.82	89.8	4.8	5.4
13	7.61	3.06	4.42	0.56	91.7	3.4	4.9
15	7.52	0.45	2.30	0.44	90.6	3.9	5.5
17	7.70	1.80	4.86	0.72	90.3	4.0	5.7
19	7.74	4.68	3.50	0.66	90.3	3.0	6.7
21	7.50	7.38	5.81	0.74	96.0	1.7	2.3
23	7.49	2.97	4.91	0.66	93.6	2.6	3.8
25	7.42	0.18	4.45	0.52	97.5	1.6	0.9
27	7.68	1.17	5.46	0.68	90.7	2.9	6.4
29	7.70	1.80	5.48	0.92	91.4	4.2	4.4
31	7.45	4.94	3.65	0.61	93.3	1.5	5.2
33	7.50	2.70	5.88	0.60	96.6	1.5	1.9
35	7.44	6.30	7.03	0.86	94.7	1.9	3.4
37	7.50	2.20	3.55	0.65	95.0	1.8	3.2

TABLE 19

Composition of Rumen Liquor Samples from Cow No. 5.

Week	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.70	6.57	1.60	71.5	39.1	12.0	1.1	16.6	0.8	1.2
3	6.68	3.06	0.97	65.0	40.0	9.3	0.7	10.6	0.8	0.6
5	6.40	1.89	0.32	78.8	48.2	11.9	0.9	12.9	0.8	1.4
7	6.58	1.17	0.46	64.4	37.5	10.0	0.8	10.5	1.0	0.6
9	6.62	1.44	0.10	72.1	43.2	10.6	0.8	11.1	0.8	0.4
11	6.40	0.45	0.20	69.5	44.0	9.6	1.1	9.6	1.3	0.8
13	6.66	0.27	0.00	78.3	46.2	12.2	1.3	14.2	1.7	1.6
15	6.55	0.09	0.09	74.6	47.3	11.6	1.3	10.2	1.7	1.3
17	6.65	2.70	0.90	73.3	44.4	11.3	0.9	12.2	1.5	0.8
19	6.70	2.70	0.00	60.5	36.9	9.1	0.9	8.3	1.1	1.3
21	6.60	0.00	0.11	68.7	44.3	10.1	0.9	8.7	0.8	0.6
23	6.60	1.80	1.17	73.1	47.5	11.1	0.7	10.0	1.0	0.8
25	6.60	1.80	1.18	70.1	44.1	10.3	1.0	10.3	1.5	0.9
27	6.62	1.17	1.05	64.8	40.8	8.9	0.8	9.9	1.2	1.0
29	6.78	5.40	0.07	65.3	41.3	8.5	1.0	9.9	1.9	0.8
31	6.79	2.70	0.14	58.8	36.7	8.5	0.7	8.6	1.1	0.6
33	6.53	2.52	1.05	75.0	48.5	12.5	1.3	10.6	1.3	1.1
35	6.80	2.25	0.68	64.6	32.8	6.9	0.9	7.7	1.3	1.2
37	6.70	1.95	0.57	68.5	44.0	10.9	1.0	10.2	1.5	0.9

TABLE 20.

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 5.

Week	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	55.2	16.9	1.5	23.5	1.2	1.7
3	64.5	15.0	1.2	17.1	1.3	0.9
5	63.4	15.6	1.2	17.0	1.1	1.7
7	62.3	16.5	1.2	17.4	1.6	1.0
9	64.5	15.8	1.2	16.6	1.2	0.7
11	66.3	14.5	1.6	14.4	2.0	1.2
13	60.1	16.0	1.7	18.7	2.2	1.3
15	64.5	15.8	1.7	14.0	2.3	1.7
17	62.5	15.9	1.2	17.1	2.1	1.2
19	64.1	15.7	1.6	14.4	1.8	2.4
21	67.8	15.4	1.4	13.3	1.2	0.9
23	66.9	15.7	1.0	14.0	1.3	1.1
25	64.8	15.1	1.5	15.1	2.2	1.3
27	65.1	14.2	1.3	15.8	2.0	1.6
29	65.2	13.4	1.6	15.5	3.1	1.2
31	65.3	15.2	1.2	15.4	2.0	0.9
33	64.3	16.6	1.7	14.1	1.8	1.5
35	64.6	13.6	1.9	15.1	2.5	2.3

TABLE 21

Yield and Composition of Milk for Cow No. 6.

Week	Yield lb.	Fat %	Solids-not-fat %	Crude Protein %	Lactose %	Chloride mg/100ml
1	45.0	4.25	9.05	3.50	4.60	110
3.	47.0	3.65	8.20	2.90	4.55	106
5	40.0	3.44	8.16	2.74	4.74	105
7	35.0	3.13	8.27	2.84	4.84	93
9	20.0	3.75	8.25	3.08	4.57	73
11	16.5	4.90	7.54	2.95	4.03	80
13	7.5	5.66	8.08	2.80	4.52	97

TABLE 22.

Composition of Blood Samples from Cow No. 6.

Week	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.63	0.56	15.37	0.75	97.2	1.6	1.2
3	7.40	0.08	16.39	0.65	93.6	3.4	3.0
5	7.63	2.97	13.71	1.80	92.8	4.5	2.7
7	7.45	0.15	7.43	0.35	95.6	1.7	2.7
9	7.42	0.24	7.38	1.60	95.0	2.2	2.8
11	7.58	0.25	21.20	0.56	96.7	1.6	1.7
13	7.60	0.00	14.35	1.90	97.0	2.0	1.0

TABLE 23

Composition of Rumen Liquor Samples from Cow No. 6.

Week	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.90	2.30	1.20	98.0	65.5	15.6	1.0	12.5	2.3	1.1
3	7.10	0.85	0.05	86.2	52.0	14.0	0.9	14.2	2.9	2.2
5	6.85	3.70	1.02	95.0	53.5	12.1	1.4	14.1	2.5	1.4
7	6.90	0.99	0.40	75.5	42.0	17.6	1.2	12.1	1.8	0.8
9	7.35	7.79	1.61	80.0	51.0	12.9	0.6	11.3	3.2	1.0
11	6.80	0.97	0.35	64.6	39.5	10.7	1.5	9.2	3.0	0.7
13	6.70	0.00	1.30	100.0	63.5	15.8	2.0	14.4	2.6	1.7

APPENDIX II

Stage 1	6 days before calving
Stage 2	5 days before calving
Stage 3	4 days before calving
Stage 4	Day of calving
Stage 5	1 day after calving
Stage 6	2 days after calving
Stage 7	8 days after calving
Stage 8	10 days after calving
Stage 9	16 days after calving

TABLE 24

Composition of Blood Samples from Cow No. 7

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.52	0.40	1.87	1.19	97.5	1.7	0.8
2	7.68	0.30	2.05	1.07	98.1	1.9	0.0
3	7.72	0.36	2.26	0.64	96.9	1.6	1.6
4	7.49	0.36	4.07	1.78	98.3	1.1	0.6
5	7.48	0.45	1.91	0.76	96.1	1.3	2.6
6	7.41	0.30	2.20	0.78	96.2	2.6	1.3
7	7.64	0.29	3.48	0.96	97.9	1.0	1.0
8	7.38	0.22	3.36	0.57	94.7	3.5	1.8
9	7.42	0.45	5.96	1.95	97.9	1.5	1.0

TABLE 25

Composition of Rumen Liquor Samples from Cow No. 7

Week	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.95	0.34	0.28	59.3	33.2	11.3	1.2	11.4	1.3	0.8
2	6.80	0.42	0.41	50.9	35.2	9.4	0.5	4.9	0.5	0.4
3	6.90	0.46	0.46	47.2	20.9	7.5	0.6	5.2	1.2	0.7
4	6.70	0.36	0.53	64.4	43.4	9.7	0.7	8.2	1.6	0.8
5	6.70	0.50	0.36	24.1	15.7	3.5	0.4	3.6	0.7	0.2
6	6.80	0.53	0.09	25.6	15.7	4.7	0.4	3.7	0.7	0.4
7	6.70	0.20	0.02	31.3	15.0	10.7	0.2	4.1	0.5	0.8
8	6.85	0.34	0.21	31.6	16.7	8.5	0.2	4.9	0.6	0.7
9	6.90	0.55	0.48	68.9	44.1	12.9	0.8	9.0	1.2	0.9

TABLE 26

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 7.

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	56.0	19.1	2.0	19.2	2.2	1.3
2	69.2	18.5	1.0	9.6	1.0	0.8
3	57.9	20.8	1.7	14.4	3.3	1.9
4	67.4	15.1	1.1	12.7	2.5	1.2
5	65.1	14.5	1.7	14.9	2.9	0.8
6	61.3	18.4	1.6	14.5	2.7	1.6
7	47.9	34.2	0.6	13.1	1.6	2.6
8	52.8	26.9	0.6	15.5	1.9	2.2
9	64.0	18.7	1.2	13.1	1.7	2.5

TABLE 27

Composition of Blood Samples from Cow No. 8.

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.45	0.70	3.89	0.76	94.7	2.6	2.6
2	7.44	0.47	4.56	1.05	95.2	2.9	1.9
3	7.52	0.53	2.55	1.04	97.1	1.0	1.9
4	7.56	0.75	5.45	1.34	97.0	1.5	1.5
5	7.53	0.49	2.58	0.86	91.9	2.3	5.8
6	7.54	0.43	3.92	0.88	94.3	3.4	2.3
7	7.65	0.79	3.85	0.95	95.8	2.1	2.1
8	7.65	0.78	5.52	0.90	95.6	2.2	2.2
9	7.45	1.04	5.96	1.70	95.8	1.2	2.9

TABLE 28

Composition of Rumen Liquor Samples from Cow No. 8

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.85	0.02	0.18	57.9	39.2	8.2	1.1	6.7	1.8	0.7
2	7.00	0.21	0.26	71.5	51.3	11.0	0.7	7.2	0.9	0.4
3	6.80	0.04	0.34	61.1	41.7	8.0	1.0	7.9	1.7	0.8
4	6.50	0.22	0.20	52.5	34.9	7.2	0.9	7.5	1.1	0.7
5	6.40	0.12	0.02	56.2	39.3	8.4	1.0	5.7	1.3	0.5
6	6.42	0.03	0.28	93.9	63.7	12.8	1.3	12.1	2.3	1.0
7	6.90	0.31	0.28	77.2	50.1	12.1	1.0	11.7	1.6	0.7
8	7.20	0.20	0.14	77.6	52.2	10.4	0.8	11.7	1.6	0.8
9	6.55	0.46	0.49	61.0	40.7	8.7	0.6	9.3	1.1	0.6

TABLE 29

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 8.

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	67.7	14.2	1.9	11.6	3.1	1.2
2	71.8	15.4	1.0	10.1	1.3	0.6
3	68.3	13.1	1.6	12.9	2.8	1.3
4	66.7	13.8	1.7	14.3	2.1	1.3
5	69.9	15.0	1.8	10.1	2.3	0.9
6	67.8	13.6	1.4	12.9	2.5	1.1
7	64.9	15.7	1.3	15.2	2.1	0.9
8	67.3	13.4	1.0	15.1	2.1	1.0
9	66.7	14.3	1.0	15.3	1.8	1.0

TABLE 30

Composition of Blood Samples from Cow No. 9

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.38	0.91	3.93	0.96	95.8	2.1	2.1
2	7.60	0.21	1.73	0.90	94.4	3.3	2.2
3	7.48	0.53	0.78	1.07	94.4	2.8	2.8
4	7.49	0.39	5.33	1.84	94.6	2.7	2.7
5	7.40	0.30	1.72	1.51	92.7	3.3	4.0
6	7.60	0.12	3.19	0.91	87.9	5.5	6.6
7	7.60	0.68	2.59	1.58	94.3	4.4	1.3
8	7.52	0.78	3.34	1.15	89.6	5.2	5.2
9	7.50	0.84	5.30	1.71	94.7	2.9	2.3

TABLE 31

Composition of Rumen Liquor Samples from Cow No. 9

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.55	0.80	0.30	64.2	38.7	11.1	0.9	11.0	1.6	0.9
2	7.20	0.26	0.29	26.0	17.9	4.6	0.3	2.5	0.5	0.2
3	6.90	0.37	0.49	12.9	11.3	1.2	0.1	0.4	0.1	0.1
4	6.35	0.29	0.47	38.1	25.3	5.5	0.6	4.8	0.8	0.9
5	6.90	0.08	0.00	63.3	55.3	10.8	1.2	12.1	1.6	0.8
6	6.32	0.01	0.00	91.4	61.7	13.5	1.0	11.9	1.7	1.0
7	6.90	0.33	0.54	76.0	47.5	11.3	0.8	13.4	1.7	1.1
8	7.03	0.24	0.47	51.5	32.7	8.2	0.7	7.7	1.2	0.7
9	6.60	0.38	0.27	70.0	49.8	11.5	0.5	7.2	0.6	0.4

TABLE 32

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 9.

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	60.3	17.3	1.4	17.1	2.5	1.4
2	68.9	17.7	1.2	9.6	1.9	0.8
3	85.6	9.1	0.8	3.0	0.8	0.8
4	66.4	14.4	1.6	12.6	2.1	2.4
5	67.6	13.2	1.5	14.8	2.0	1.0
6	67.5	14.8	1.1	13.0	1.9	1.1
7	62.5	14.9	1.1	17.6	2.2	1.5
8	63.5	15.9	1.4	15.0	2.3	1.4
9	71.1	16.4	0.7	10.3	0.9	0.6

TABLE 33

Composition of Blood Samples from Cow No. 10

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.50	0.56	2.46	0.81	92.6	2.5	6.0
2	7.52	0.40	3.07	0.78	94.9	2.6	2.6
3	7.39	0.36	2.82	0.68	97.1	1.5	1.5
4	7.60	0.00	5.05	0.98	96.9	2.0	1.0
5	7.41	0.31	1.85	0.65	93.9	1.5	4.6
6	7.60	0.38	2.60	0.76	94.7	2.6	2.6
7	7.67	0.53	3.87	0.87	95.5	1.1	3.4
8	7.52	0.74	3.53	0.80	96.3	1.3	2.5
9	7.50	0.30	4.41	1.30	86.8	1.1	11.8

TABLE 34

Composition of Rumen Liquor Samples from Cow No. 10

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.90	0.35	0.25	82.3	59.7	12.0	0.7	8.4	0.9	0.6
2	6.85	0.45	0.63	87.5	56.1	16.8	1.3	9.7	2.4	1.1
3	7.00	0.00	0.56	90.8	62.8	13.5	1.0	11.9	1.0	0.6
4	7.35	0.00	0.33	52.5	32.7	7.8	0.4	8.7	1.8	1.1
5	7.38	0.12	0.35	46.0	28.5	7.6	0.6	7.6	0.9	0.8
6	6.74	0.15	0.15	65.0	40.0	11.2	0.5	12.6	0.7	0.8
7	6.80	0.40	0.06	94.8	67.3	11.5	1.1	13.3	0.8	0.8
8	6.52	0.30	0.36	97.0	67.2	16.8	1.3	9.3	1.8	0.6
9	6.75	0.20	0.41	86.0	55.0	16.2	1.0	10.7	1.5	1.6

TABLE 35

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 10

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	72.5	14.6	0.9	10.2	1.1	0.7
2	64.1	19.2	1.5	11.1	2.7	1.3
3	69.2	14.9	1.1	13.1	1.1	0.7
4	62.3	14.9	0.8	16.6	3.4	2.1
5	62.0	16.5	1.3	16.5	2.0	1.7
6	60.8	17.0	0.8	19.2	1.1	1.2
7	71.0	12.1	1.2	14.0	0.8	0.8
8	69.3	17.3	1.3	9.6	1.9	0.6
9	64.0	18.9	1.2	12.4	1.7	1.9

TABLE 36

Composition of Blood Samples from Cow No. 11

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.60	0.27	2.31	0.65	93.9	1.5	4.6
2	7.42	0.26	2.41	0.70	92.9	2.9	4.3
3	7.42	0.35	1.64	0.81	93.8	2.5	3.7
4	7.40	0.16	3.78	1.02	95.1	2.0	3.0
5	7.45	0.22	2.85	0.68	92.7	2.9	4.4
6	7.50	0.07	2.05	0.95	89.5	2.1	8.4
7	7.48	0.27	2.65	0.86	93.0	4.6	2.3
8	7.55	0.40	3.01	0.91	91.2	4.4	4.4
9	7.46	0.07	6.57	1.41	96.5	2.1	1.4

TABLE 37

Composition of Rumen Liquor Samples from Cow No. 11

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	7.10	0.18	0.39	92.5	68.5	11.9	1.0	9.1	1.5	0.5
2	6.80	0.22	0.20	87.5	65.0	11.4	0.5	9.3	0.8	0.5
3	6.95	0.25	0.08	93.3	67.4	12.8	0.9	10.3	1.2	0.7
4	7.10	0.20	0.56	69.0	50.3	9.4	0.8	7.0	1.0	0.5
5	6.89	0.05	0.37	72.1	53.9	10.6	0.7	6.1	0.5	0.3
6	6.94	0.15	0.62	76.2	53.7	12.2	0.9	7.6	1.1	0.7
7	7.00	0.26	0.25	80.0	52.0	12.6	1.1	8.5	1.5	0.9
8	6.85	0.30	0.09	76.0	52.5	9.9	0.9	10.3	1.2	0.7
9	7.15	0.00	0.05	69.0	52.0	8.4	0.7	7.1	0.6	0.6

TABLE 38

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 11

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	74.1	12.9	1.1	9.8	1.6	0.5
2	74.3	13.0	0.6	10.6	0.9	0.6
3	72.2	13.7	1.0	11.0	1.3	0.8
4	72.9	13.6	1.2	10.1	1.5	0.7
5	74.8	14.7	1.0	8.5	0.7	0.4
6	70.5	16.0	1.2	10.0	1.4	0.9
7	67.9	16.5	1.4	11.1	2.0	1.2
8	69.1	13.0	1.2	13.6	1.6	0.9
9	75.4	12.2	1.0	10.3	0.9	0.9

TABLE 39

Composition of Blood Samples from Cow No. 12

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.41	0.86	2.96	0.76	94.7	1.3	4.0
2	7.40	0.04	2.07	0.65	95.4	3.1	1.5
3	7.50	0.26	2.23	0.59	96.6	1.7	1.7
4	7.47	0.16	5.86	0.78	94.9	2.6	2.6
5	7.45	0.28	2.65	1.02	95.1	2.0	2.9
6	7.55	0.19	1.06	0.69	95.7	2.9	1.5
7	7.50	0.35	4.75	0.76	96.1	2.6	1.3
8	7.42	0.17	4.56	0.82	93.9	2.4	3.7
9	7.40	0.23	10.23	1.67	95.2	3.0	1.8

TABLE 40

Composition of Rumen Liquor Samples from Cow No. 12

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.80	0.50	0.60	77.8	51.5	9.5	0.9	9.9	1.2	0.7
2	6.65	0.25	0.09	64.3	38.0	11.0	0.9	11.5	1.6	1.1
3	6.70	0.40	0.56	70.5	52.5	8.8	0.8	7.6	0.6	0.6
4	7.38	0.00	0.30	40.6	29.3	4.0	0.9	4.1	0.7	1.0
5	7.37	0.05	0.25	59.8	33.0	11.5	1.4	11.7	1.2	0.9
6	6.90	0.35	0.42	72.0	44.6	13.5	0.8	12.0	0.6	0.5
7	7.01	0.55	0.28	76.2	52.3	10.3	0.9	9.8	1.3	1.2
8	6.60	0.20	0.03	69.7	51.0	10.2	0.9	5.9	1.1	0.6
9	6.80	0.06	0.44	80.5	51.0	12.9	1.1	13.0	1.3	1.2

TABLE 41.

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No.12.

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	69.9	12.9	1.2	13.4	1.6	1.0
2	59.1	17.1	1.4	17.9	2.5	1.7
3	74.5	12.5	1.1	10.8	0.9	0.9
4	73.3	10.0	2.3	10.3	1.8	2.5
5	55.2	19.2	2.3	19.6	2.0	1.5
6	61.9	18.8	1.1	16.7	0.8	0.7
7	68.6	13.5	1.2	12.9	1.7	1.6
8	73.2	14.6	1.3	8.5	1.6	0.9
9	63.3	16.0	1.4	16.2	1.6	1.5

TABLE 42

Composition of Blood Samples from Cow No. 13

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.60	0.43	2.75	0.59	94.9	1.7	3.4
2	7.43	0.40	3.32	0.55	96.3	1.9	1.8
3	7.47	0.34	3.25	0.58	94.9	3.4	1.7
4	7.39	0.00	5.76	0.79	94.9	2.5	2.5
5	7.62	0.12	2.05	0.51	96.1	2.0	2.0
6	7.58	0.14	1.95	0.43	95.4	4.7	0.0
7	7.44	0.50	3.76	0.72	94.4	4.2	1.4
8	7.39	0.16	4.02	0.66	95.5	1.5	3.0
9	7.50	0.31	7.95	0.96	95.8	1.0	3.1

TABLE 43

Composition of Rumen Liquor Samples from Cow No. 13

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.90	0.33	0.45	122.6	92.6	14.7	0.8	12.5	1.2	0.5
2	6.65	0.21	0.55	81.1	60.6	10.0	0.7	8.7	0.5	0.3
3	6.87	0.16	0.30	84.7	61.9	12.6	0.8	8.1	0.8	0.6
4	7.30	0.00	0.06	84.7	62.5	12.0	0.7	8.2	0.7	0.5
5	6.90	0.22	0.44	75.6	47.1	11.3	0.8	13.3	1.6	1.2
6	7.08	0.29	0.18	71.3	49.5	12.5	0.5	7.8	0.6	1.0
7	6.70	0.25	0.21	87.6	60.4	15.2	0.9	9.5	0.8	0.9
8	7.07	0.05	0.48	64.3	38.0	11.0	0.9	11.5	1.6	1.0
9	6.90	0.12	0.09	67.8	48.0	10.3	0.5	7.0	0.6	1.2

TABLE 44

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 13

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	75.7	12.0	0.7	10.3	1.0	0.4
2	74.7	12.3	0.9	10.7	0.6	0.7
3	73.1	14.9	0.9	9.6	0.9	0.6
4	73.8	14.2	0.8	9.7	0.8	0.7
5	62.5	15.0	1.1	17.6	2.1	1.7
6	69.4	17.5	0.7	10.9	0.8	0.6
7	69.0	17.4	1.0	10.8	0.9	0.9
8	59.3	17.2	1.4	17.9	2.5	1.7
9	70.8	15.2	0.7	10.3	0.9	2.1

TABLE 45

Composition of Blood Samples from Cow No. 14

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.60	0.00	2.95	0.57	96.5	1.8	1.8
2	7.64	0.10	1.25	0.86	94.2	2.3	3.5
3	7.72	0.12	2.78	0.77	94.8	2.6	2.6
4	7.49	0.12	3.88	0.70	95.7	1.4	2.9
5	7.48	0.10	2.30	1.08	96.3	1.9	1.9
6	7.41	0.20	2.93	0.96	96.9	2.1	1.0
7	7.64	0.16	2.93	1.06	96.2	1.9	1.9
8	7.38	0.21	3.86	1.15	95.7	0.9	2.6
9	7.42	0.05	5.96	1.32	93.9	3.8	2.3

TABLE 46

Composition of Rumen Liquor Samples from Cow No. 14

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.60	0.05	0.57	69.5	45.2	12.8	0.5	10.0	0.4	0.6
2	6.80	0.10	0.34	70.2	45.2	12.0	1.0	10.3	0.9	0.8
3	7.00	0.15	0.21	68.0	44.2	11.5	1.2	8.3	1.1	0.7
4	6.80	0.05	0.25	67.6	42.8	10.0	0.9	10.8	1.3	1.8
5	6.75	0.12	0.35	82.8	51.4	15.2	1.4	12.2	0.8	1.7
6	6.60	0.15	0.30	72.0	45.8	12.8	0.9	10.5	1.4	0.6
7	7.10	0.16	0.42	65.6	43.0	9.0	1.4	9.1	1.0	2.1
8	6.50	0.09	0.40	72.0	48.7	12.5	1.1	8.2	0.8	0.7
9	6.75	0.18	0.35	70.0	44.3	11.9	1.4	10.0	1.6	0.8

TABLE 47.

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 14.

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	65.0	18.4	0.7	14.4	0.6	0.9
2	64.4	17.1	1.4	14.7	1.3	1.1
3	66.0	17.2	1.8	12.4	1.6	1.0
4	63.3	14.8	1.3	16.0	1.9	2.7
5	62.2	18.4	1.7	14.8	1.0	2.1
6	63.6	17.8	1.3	14.6	1.9	0.8
7	65.6	13.7	2.1	13.9	1.5	3.2
8	67.6	17.4	1.5	11.4	1.1	1.0
9	63.3	17.0	2.0	14.3	2.3	1.1

TABLE 48

Composition of Blood Samples from Cow No. 15

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.50	1.30	2.69	0.71	95.8	1.4	2.8
2	7.65	0.40	2.85	0.75	93.3	4.0	2.7
3	7.42	0.03	3.05	0.71	94.4	2.8	2.8
4	7.55	0.15	4.96	0.96	96.9	1.0	2.1
5	7.62	0.27	2.06	0.58	94.8	3.5	1.7
6	7.60	0.25	3.21	0.56	96.4	1.8	1.8
7	7.61	0.03	4.05	0.76	96.1	2.6	1.3
8	7.52	0.32	3.96	0.75	94.7	1.3	4.0
9	7.70	0.16	6.75	1.05	96.2	1.9	1.9

TABLE 49

Composition of Rumen Liquor Samples from Cow No. 15.

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.60	0.26	0.60	66.5	44.7	12.0	0.7	7.1	1.2	0.8
2	6.90	0.37	0.75	70.0	44.3	9.5	0.6	13.7	1.0	0.9
3	6.70	0.25	0.42	68.2	47.6	10.4	0.8	7.6	1.2	0.6
4	6.80	0.02	0.42	35.0	25.2	3.5	0.6	4.1	0.8	0.8
5	6.90	0.15	0.15	39.6	28.9	4.4	0.7	3.7	0.9	1.0
6	7.20	0.12	0.17	72.1	44.7	12.0	0.6	13.5	0.8	0.5
7	6.90	0.36	0.85	75.0	48.8	13.3	1.0	10.1	0.9	0.9
8	6.70	0.20	1.36	74.1	49.4	13.0	0.8	9.6	0.8	0.5
9	6.60	0.20	0.43	70.5	46.2	9.2	1.0	10.3	1.1	0.7

TABLE 50

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No.15.

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	67.2	18.1	1.1	10.7	1.8	1.2
2	63.3	13.6	0.9	19.6	1.4	1.3
3	69.8	15.3	1.2	11.1	1.8	0.9
4	72.0	10.0	1.7	11.7	2.3	2.3
5	73.0	11.1	1.8	9.3	2.3	2.5
6	62.0	16.6	0.8	18.7	1.1	0.7
7	65.1	17.7	1.3	13.5	1.2	1.2
8	66.7	17.5	1.1	13.0	1.1	0.7
9	67.5	13.4	1.5	15.0	1.6	1.0

APPENDIX III

Stage 1	4 days before drying-off
Stage 2	3 days before drying-off
Stage 3	2 days before drying-off
Stage 4	1 day after drying-off
Stage 5	3 days after drying-off
Stage 6	5 days after drying-off
Stage 7	9 days after drying-off
Stage 8	11 days after drying-off
Stage 9	17 days after drying-off

TABLE 51

Composition of Blood Samples from Cow No. 16.

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.60	0.51	1.45	1.08	98.2	0.9	0.9
2	7.70	0.43	1.34	1.00	98.0	2.0	0.0
3	7.49	0.23	0.35	1.18	97.5	1.7	0.9
4	7.80	0.33	1.52	0.93	98.9	1.1	0.0
5	7.81	0.47	1.08	0.91	98.9	1.1	0.0
6	7.03	0.18	1.89	1.16	97.4	1.7	0.9
7	7.45	0.13	0.94	1.66	97.0	1.8	1.2
8	7.48	1.29	1.09	1.43	94.4	3.5	2.1
9	7.52	0.24	1.16	0.88	97.7	1.1	1.1

TABLE 52

Composition of Rumen Liquor Samples from Cow No. 16

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.75	0.37	0.34	87.1	60.9	11.3	0.5	12.3	1.2	0.9
2	6.95	0.24	0.32	86.0	59.8	13.8	0.5	10.3	0.7	0.9
3	6.21	0.23	0.37	89.8	59.0	14.2	0.7	13.5	1.2	1.2
4	6.95	0.31	0.32	71.1	48.4	9.2	1.0	10.5	1.3	0.7
5	7.19	0.17	0.25	42.6	29.9	5.6	0.4	5.3	0.9	0.5
6	6.18	0.06	0.61	77.2	53.4	12.1	0.6	9.7	0.8	0.6
7	6.71	0.21	0.17	67.3	45.9	8.9	0.9	9.6	1.4	0.6
8	6.59	0.71	0.23	65.5	45.8	9.9	0.6	7.1	1.4	0.7
9	6.70	0.28	0.37	85.8	58.8	11.4	1.2	11.3	2.3	0.8

TABLE 53

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 16

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	69.9	13.0	0.6	14.1	1.4	1.0
2	69.5	16.1	0.5	12.0	0.9	1.0
3	65.7	15.8	0.8	15.0	1.3	1.4
4	68.1	13.0	1.4	14.8	1.9	0.8
5	70.2	13.2	0.9	12.4	2.1	1.2
6	69.2	15.7	0.8	12.6	1.0	0.7
7	68.2	13.2	1.3	14.3	2.1	0.9
8	69.9	15.1	0.9	10.8	2.1	1.2
9	68.5	13.3	1.4	13.2	2.7	0.9

TABLE 54

Composition of Blood Samples from Cow No. 17

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.55	0.52	1.12	0.98	98.0	1.0	1.0
2	7.77	0.38	0.50	1.23	97.6	1.6	0.8
3	7.37	0.31	1.48	1.59	98.7	1.3	0.0
4	7.90	0.36	1.13	1.17	99.2	0.9	0.0
5	7.71	0.11	0.80	0.72	98.6	1.4	0.0
6	6.94	0.18	0.74	0.99	98.0	2.0	0.0
7	7.40	0.15	0.94	1.32	96.2	2.3	1.5
8	7.44	0.24	0.88	1.12	97.3	1.8	0.9
9	7.45	0.34	1.46	1.54	98.1	1.3	0.7

TABLE 55

Composition of Rumen Liquor Samples from Cow No.17

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace- tone	Beta- hydroxy- butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso- Butyric Acid	Butyric Acid	Iso- Valeric Acid	Valeric Acid
1	6.30	0.44	0.28	101.5	71.8	14.0	0.8	11.9	2.1	0.9
2	6.80	0.22	0.32	85.3	60.6	11.8	0.6	10.6	1.1	0.8
3	6.50	0.27	0.31	87.3	61.1	11.7	0.6	11.4	1.6	0.9
4	7.36	0.47	0.62	56.9	40.4	7.5	0.7	6.8	1.1	0.4
5	7.40	0.11	0.18	61.2	42.8	8.2	0.5	7.4	1.6	0.4
6	6.25	0.09	0.50	74.3	54.0	10.6	0.5	7.3	1.3	0.6
7	6.92	0.51	0.14	72.5	51.1	10.5	0.6	8.6	1.1	0.6
8	6.75	0.39	0.29	73.5	52.2	10.7	0.6	8.2	1.3	0.8
9	6.90	0.50	0.24	85.1	58.4	11.8	1.3	11.0	2.2	0.4

TABLE 56

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No.17

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	70.8	13.8	0.8	11.7	2.1	0.8
2	70.9	13.9	0.7	12.4	1.3	0.8
3	70.0	13.4	0.7	13.1	1.8	1.0
4	71.0	13.2	1.2	12.0	1.9	0.7
5	69.9	13.4	0.8	12.1	2.6	1.2
6	72.7	14.3	0.7	9.8	1.8	0.7
7	70.5	14.5	0.8	11.9	1.5	0.8
8	70.8	14.5	0.9	11.2	1.7	0.9
9	68.1	13.8	1.5	12.8	2.6	1.2

TABLE 57

Composition of Blood Samples from Cow No. 18.

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.60	0.49	1.55	0.96	99.0	1.0	0.0
2	7.70	0.49	2.04	1.18	98.3	1.7	0.0
3	7.43	0.34	1.55	1.20	99.2	0.8	0.0
4	7.50	0.27	2.08	1.04	98.1	1.0	1.0
5	7.49	0.04	0.85	0.81	97.5	1.2	2.5
6	7.49	0.05	1.34	1.34	98.5	0.8	0.8
7	7.50	0.27	1.15	1.35	96.3	2.2	1.5
8	7.65	0.27	1.01	0.93	95.7	2.2	2.2
9	7.50	0.31	1.55	0.85	97.7	1.2	1.2

TABLE 58

Composition of Rumen Liquor Samples from Cow No. 18

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	7.10	0.40	0.31	83.7	56.4	11.0	0.9	12.9	1.5	1.0
2	6.90	0.21	0.47	67.2	47.3	8.6	0.6	9.1	0.9	0.7
3	6.35	0.33	0.12	93.6	65.7	12.4	0.6	13.1	0.9	0.9
4	6.75	0.23	0.37	55.9	37.8	7.3	1.0	8.2	1.0	0.7
5	6.75	0.07	0.18	52.6	35.3	8.0	0.7	6.8	1.1	0.7
6	6.00	0.05	0.56	62.0	43.7	8.3	0.3	8.7	0.5	0.6
7	6.50	0.28	0.07	75.8	51.8	10.4	0.9	10.7	1.1	0.9
8	6.75	0.24	0.73	80.7	55.4	13.3	1.2	8.2	1.7	0.9
9	7.25	0.39	0.27	60.2	37.9	8.3	1.4	9.8	1.9	0.9

TABLE 59

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 18

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	67.4	13.1	1.1	15.4	1.8	1.2
2	70.4	12.8	0.9	13.5	1.3	1.1
3	70.2	13.3	0.6	14.0	1.0	0.9
4	67.6	13.1	1.8	14.6	1.8	1.1
5	67.3	15.2	1.3	13.0	1.9	1.3
6	70.6	12.9	0.5	14.1	0.9	1.0
7	68.3	13.7	1.2	14.1	1.5	1.2
8	68.7	16.5	1.5	10.2	2.1	1.0
9	63.0	13.8	2.3	16.3	3.2	1.4

TABLE 60

Composition of Blood Samples from Cow No. 19

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.60	0.69	2.72	1.20	98.3	0.8	0.8
2	7.58	0.19	1.62	1.15	98.3	0.9	0.9
3	7.65	0.90	3.85	1.10	99.1	0.9	0.0
4	7.78	0.40	2.22	0.80	97.5	1.3	1.3
5	7.70	0.27	2.08	0.65	98.5	1.5	0.0
6	7.80	0.48	2.17	1.25	96.0	1.6	2.4
7	7.75	0.30	2.00	1.13	97.4	1.8	0.9
8	7.68	0.17	1.76	1.00	99.0	1.0	0.0
9	7.70	0.80	1.90	0.85	97.7	1.2	1.2

TABLE 61

Composition of Rumen Liquor Samples from Cow No. 19

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.20	0.12	0.49	82.7	60.0	10.5	0.7	10.0	0.9	0.6
2	6.95	0.37	0.55	63.6	46.2	8.0	0.5	7.7	0.7	0.5
3	6.80	0.23	0.61	74.7	54.9	8.4	0.6	9.5	0.8	0.5
4	6.95	0.26	0.56	71.3	51.7	9.0	0.6	8.6	0.8	0.6
5	6.25	0.09	0.39	83.0	61.0	10.0	0.6	10.2	0.8	0.6
6	6.80	0.31	0.48	63.8	47.0	7.8	0.5	7.5	0.6	0.4
7	6.10	0.28	0.34	87.7	63.6	11.1	0.7	10.6	1.0	0.7
8	6.85	0.39	0.42	68.5	50.0	8.5	0.5	8.2	0.7	0.6
9	7.01	0.46	0.50	75.1	55.8	8.9	0.7	8.4	0.7	0.6

TABLE 62

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 19

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	72.6	12.7	0.9	12.1	1.1	0.6
2	72.6	12.6	0.8	12.1	1.1	0.8
3	73.5	11.2	0.8	12.7	1.1	0.7
4	72.5	12.6	0.8	12.1	1.1	0.9
5	73.3	12.0	0.7	12.3	1.0	0.7
6	73.7	12.2	0.8	11.8	0.9	0.6
7	72.5	12.7	0.8	12.1	1.1	0.8
8	73.0	12.4	0.7	12.0	1.0	0.9
9	74.3	11.9	0.9	11.2	0.9	0.8

TABLE 63

Composition of Blood Samples from Cow No. 20

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.90	0.71	2.19	1.19	98.3	0.8	0.8
2	7.63	0.56	2.17	1.34	95.5	2.2	2.2
3	7.70	0.51	3.55	1.26	97.6	1.6	0.8
4	7.75	0.41	2.92	0.82	96.3	2.4	1.2
5	7.70	0.38	1.90	1.35	95.6	1.5	3.0
6	7.80	0.01	1.32	1.16	97.4	1.7	0.9
7	7.63	0.15	1.55	1.10	98.2	1.8	0.0
8	7.75	0.46	1.76	1.30	98.5	0.8	0.8
9	7.75	0.49	1.90	1.36	96.3	2.9	0.7

TABLE 64

Composition of Rumen Liquor Samples from Cow No. 20

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.90	0.12	0.49	85.8	62.5	11.0	0.7	10.1	0.9	0.6
2	6.50	0.25	0.56	87.8	63.7	10.8	0.6	11.2	0.8	0.7
3	6.90	0.22	0.40	89.5	65.2	12.6	0.9	9.3	0.8	0.7
4	6.89	0.26	0.38	83.5	61.4	11.2	0.7	8.7	0.9	0.6
5	6.60	0.13	0.43	85.3	61.0	14.7	1.3	6.0	1.6	0.8
6	6.80	0.31	0.25	80.5	58.8	10.8	0.8	8.8	0.7	0.6
7	6.85	0.18	0.40	79.5	59.1	10.5	0.7	8.1	0.6	0.5
8	6.75	0.29	0.35	82.0	59.9	11.7	0.6	8.1	0.9	0.8
9	6.75	0.25	0.45	84.5	61.8	12.6	0.9	7.5	1.0	0.7

TABLE 65

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 20

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	72.8	12.8	0.8	11.8	1.1	0.7
2	72.6	12.3	0.7	12.8	1.0	0.6
3	72.9	14.1	1.0	10.4	0.9	0.7
4	73.5	13.4	0.8	10.4	1.1	0.8
5	71.5	17.2	1.5	7.0	1.9	0.9
6	73.0	13.4	1.0	10.9	0.9	0.8
7	74.3	13.2	0.9	10.2	0.8	0.6
8	73.1	14.3	0.7	9.9	1.1	0.9
9	73.1	14.9	1.1	8.9	1.2	0.8

TABLE 66

Composition of Blood Samples from Cow No. 21

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.90	0.47	2.45	1.20	98.3	0.8	0.8
2	7.60	0.31	1.93	1.05	99.1	1.0	0.0
3	7.65	0.35	3.41	1.15	96.5	1.7	0.9
4	7.72	0.38	1.83	0.70	98.6	1.4	0.0
5	7.54	0.37	2.28	0.90	97.8	1.1	1.1
6	7.79	0.31	1.82	1.09	96.3	1.8	1.8
7	7.80	0.35	1.95	0.80	98.8	0.0	1.3
8	7.62	0.29	2.10	1.15	97.4	1.7	0.9
9	7.74	0.40	2.35	1.02	98.0	1.0	1.0

TABLE 67

Composition of Rumen Liquor Samples from Cow No.21

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.90	0.26	0.80	86.2	63.4	10.9	1.1	9.2	1.2	0.4
2	6.56	0.20	0.64	74.0	54.0	9.9	0.6	8.4	0.6	0.5
3	6.85	0.33	0.71	74.0	53.5	9.7	0.8	8.8	0.9	0.3
4	7.00	0.30	0.57	71.6	54.7	8.8	0.5	7.1	0.3	0.2
5	7.10	0.08	0.19	77.8	59.4	9.6	0.7	7.0	0.6	0.5
6	6.95	0.36	0.62	68.4	49.5	8.9	0.6	8.4	0.5	0.5
7	6.80	0.25	0.60	69.8	50.9	9.3	0.7	7.6	0.6	0.7
8	6.78	0.28	0.50	73.0	53.7	9.2	0.6	8.3	0.5	0.7
9	6.92	0.32	0.65	70.5	53.5	8.0	0.7	7.0	0.4	0.6

TABLE 68

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 21

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	73.6	12.7	1.3	10.7	1.4	0.3
2	73.0	13.4	0.8	11.4	0.8	0.6
3	72.3	13.1	1.1	11.9	1.2	0.4
4	76.4	12.3	0.7	9.9	0.4	0.3
5	76.4	12.3	0.9	9.0	0.8	0.6
6	72.4	13.0	0.9	12.3	0.7	0.7
7	72.9	13.3	1.0	10.9	0.9	1.0
8	73.6	12.6	0.8	11.4	0.7	0.9
9	75.9	11.4	1.0	9.9	0.6	1.2

TABLE 69

Composition of Blood Samples from Cow No. 22

Stage	pH	mg.Acetone/100ml.		m.mole/litre Total Volatile Fatty Acids	Molar Percentage		
		Acetone	Beta hydroxy-butyric Acid		Acetic Acid	Propionic Acid	Butyric Acid
1	7.65	0.45	2.09	1.32	97.7	1.5	0.8
2	7.60	0.33	2.65	1.26	96.8	1.6	1.6
3	7.55	0.38	2.47	0.96	99.0	1.0	0.0
4	7.69	0.28	1.92	0.96	96.8	1.0	2.1
5	7.65	0.24	1.08	0.75	97.3	1.3	1.3
6	7.82	0.09	1.52	1.26	98.4	0.8	0.8
7	7.65	0.12	1.42	0.90	98.9	1.1	0.0
8	7.58	0.28	2.26	1.03	97.1	1.9	1.0
9	7.70	0.35	2.14	1.22	98.4	0.8	0.8

TABLE 70

Composition of Rumen Liquor Samples from Cow No.22

Stage	pH	mg acetone/ 100ml		m.mole/litre						
		Ace-tone	Beta-hydroxy-butyric Acid	Total Volatile Fatty Acids	Acetic Acid	Propionic Acid	Iso-Butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	6.86	0.29	0.45	79.5	55.8	10.5	0.5	11.1	0.8	0.8
2	6.90	0.25	0.46	73.0	48.9	11.1	1.0	9.4	1.5	1.1
3	7.10	0.27	0.41	81.1	54.8	10.6	1.5	11.9	1.5	0.8
4	7.05	0.31	0.45	76.7	51.7	10.1	0.8	11.8	1.4	0.9
5	6.80	0.22	0.26	73.9	52.0	9.5	0.7	10.0	1.0	0.7
6	6.65	0.20	0.44	78.2	53.5	10.7	0.9	11.0	1.1	1.0
7	6.90	0.29	0.35	75.1	52.9	10.0	0.4	10.5	0.6	0.7
8	6.95	0.38	0.44	79.4	50.0	11.0	1.8	12.9	2.5	1.2
9	7.10	0.37	0.46	80.7	54.8	13.6	1.3	8.4	1.7	0.9

TABLE 71

Proportion of Volatile Fatty Acids in Rumen Liquor of Cow No. 22

Stage	Molar Percentage of Total Volatile Fatty Acids					
	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Iso-Valeric Acid	Valeric Acid
1	70.2	13.2	0.6	14.0	1.0	1.0
2	67.0	15.2	1.4	12.9	2.1	0.4
3	67.6	13.1	1.9	14.7	1.9	0.8
4	67.4	13.2	1.0	15.4	1.8	1.2
5	70.4	12.9	1.0	13.5	1.4	0.8
6	68.4	13.7	1.2	14.1	1.4	1.2
7	70.4	13.3	0.5	14.0	0.8	1.0
8	63.0	13.9	2.3	16.3	3.2	1.3
9	67.9	16.9	1.6	10.4	2.1	1.1

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